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THE MECHANISM OF EVOLUTION IN LEPTINOTARSA

BY
WILLIAM LAWRENCE TOWER

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PREFACE.

The data and conclusions presented in part in this volume are the product of a project in which it was attempted to attack the "evolution problem" in one set of organisms from a number of different aspects. Evolution in organisms must be conceived of as the product of the interaction of internal and external factors, operating in strict mechanistic manner, as in non-vital phenomena, so that the central problem was the determination and proof of the mechanism of action of these two groups of factors whose operations are productive of evolutionary changes. Experimental analysis of the problems, prosecuted with rigor and thoroughness, has been held to be the only means of progress. Before this ideal could be applied much preliminary clearing of the ground had to be done, and to this I gave the necessary attention in a report published under the title "An Investigation of Evolution in Chrysomelid Beetles of the Genus Leptinotarsa."[1]

The results of the project have been the development of a mechanistic conception of evolution based upon the interplay of genetic and environic factors and the demonstration that the methods of evolution are heterogeneous, even in one group of organisms. The basis of all methods of change is found to be directly the product of the nature of the genetic factors of composition and their capacity for diverse modes of reaction, especially with factors of the environic complex. Purpose, utility, and kindred concepts have found no support, every change appearing as the chance mechanistic product of the reacting agents; while the product of the reaction either was able or not able to operate under the conditions of origination, so that survival is decided at once and not after long and faltering trials.

The project as organized consists of three parts, each necessary to the success of the others and to the hope of arriving at a knowledge of the mechanism of evolution that is not based upon plausibility and the arrangement of isolated facts upon the fabric of preconceived theory.

The first stage (reported upon in the publication referred to) must deal with the discovery and testing of some limited set of organisms in nature that are possible subjects for study and especially for the experimental portions of the investigation. Further, it is necessary to become fully acquainted with the organisms in nature and to make preliminary tests to discover whether it is possible to produce permanent changes in the material and the probable success, if any, that might be expected from an experimental attack upon this portion of the problem.

The second portion, given a successful outcome of the first, must comprise a rigorous experimental investigation of the factors of evolution and an attempt to create new attributes and qualities not existing in nature in the materials, and to discover the relation of these to old characters and their interaction when brought into combination or into competition with existing characters. With this vantage gained, it will then be possible to create in the laboratory new

[1] Carnegie Inst. Wash., Pub. 48, 330 pp., 30 pl., 31 text figs., 1906.

specific qualities and attributes, and with these and the old to create an array of new types, new genera, or even of higher taxonomic groups of organisms, and with this as a basis it is possible to attempt the third part of the project.

This last part involves experimenting in nature with new forms to discover how newly arisen characters, or their combination into specific forms, behave as they meet the conditions of the environment into which they are thrust by the processes of their origination. It is in this way only that it may be possible to discover methods of elimination, of preservation, of adaptation, and the study of kindred problems, that are of vital import in the total evolutionary activities in nature, and which are now the delight of the essayist.

Throughout this work "factor" and "determiner" are used in a physical and dynamic sense, and are nowhere used in the sense of a carrier, representative or otherwise, of anything pertaining to the characters of organisms. The word "factor," an agent that makes possible a general result, has been too often used by some Neo-Mendelians in the sense of a carrier, but I am not able to discover that this use (in the Weismannian sense) is justified, and investigations of the Mendelian reaction do justify the use of the term in the physical sense in which I have used it. A "determiner," on the other hand, is an agent which by its interaction with a factor decides which one out of several possible results shall ensue.

The presence of reactions involving these gametic agents in diverse aspects of the same materials, the experiments showing that the factors are specific in reaction only when present in a specific system and environment, are the reasons for the attempt to formulate these results in a general hypothesis of evolutionary action and causation. Unlike other evolution hypotheses any dynamic conception must be heterogeneous in action and result, a complex of many factors and types of reaction and nowhere due to the operation of a single agency. I have found in my materials that transmutation may be by sudden changes, by slow accumulation, by hybrid reaction, and by means of environmental forces, and in all changes the basal operations involved are purely physical types of reaction between the gametic agents and the condition surrounding the reaction.

I have been fortunate in the support that this project has been given. Soon after the start it was given added impetus through aid from the Elizabeth Thompson Science Fund; later the Carnegie Institution of Washington gave sundry grants for some of the investigations in the tropics, and in the later years larger and continued support at the Desert Laboratory in Tucson, Arizona, and at the Laboratory of Marine Biology at Tortugas, Florida. Also the University of Chicago has liberally supported the project, and especially the experimental portions of the investigation requiring controlled and exact conditions. I am deeply indebted to President H. P. Judson for most cordial support in the project, and to the late Professor C. O. Whitman, and especially to my colleague, Professor F. R. Lillie, for many kind acts and aid which in divers ways have contributed to the forwarding of the investigation. To President R. S. Woodward, of the Carnegie Institution of Washington, and Dr. D. T. MacDougal, Director of the Desert Laboratory at Tucson, Arizona, is due the credit for an opportunity to conduct upon an unusually extensive scale and, under favorable conditions, a series of experimental studies in the Arizona deserts. The creation of an elaborate plant with trained assistants has given me the best opportunity to experiment direct in nature.

A difficult portion of my problems has received the sympathy and aid of the Department of Marine Biology of the Carnegie Institution of Washington, and its Director, Dr. A. G. Mayer, has spared no pains and expense in the attempts to stock with alien races the coral islands of the Tortugas and Marquesas groups in the effort to learn, by experiment in nature, something of the laws of evolution, by dispersion, isolation, and changed environment.

It is also a pleasure to acknowledge the helpful and loyal aid received from many others in the progress of this investigation, and especially I am indebted to Dr. J. K. Breitenbecher and Mr. J. G. Sinclair for enthusiastic aid in the Tucson experiments.

That portion of the project which from 1904 to 1910 was conducted in the tropical regions of Mexico, and especially in the rain forests of Vera Cruz, has been materially aided by many friends in that country. Especially I am indebted to the late James Parkin, of the Hacienda Motzorongo, and to his administratores, Señor P. Randolph and Señor I. P. Lorio, for valuable aid in much of the transplantation and acclimatization experiments in the rain forests of Vera Cruz. These friends and the patient, loyal Indian helpers and packers made possible a series of complex experiments in the midst of a virgin tropical rain forest.

In the preparation of the material for publication, much of the reduction of data and compilation of the tables has been done by Miss Edna Scott. The drawings in color, the colored photographs, and all of the prints of negatives taken in the field, as well as the text illustrations, have been prepared by Mr. K. Toda.

To these friends and to many others I am under obligations for many kindly acts in aid of this investigation, which have been contributed to its good points, but to me must be charged that which proves to be error or misdirected effort. No finality is expected and I shall feel that my efforts have not been in vain if either data or hypotheses serve to stimulate further advances or help to correct present misconceptions.

WILLIAM LAWRENCE TOWER.

DESERT BOTANICAL LABORATORY,
November 1, 1916.

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ITHACA, N. Y.

CHAPTER I.

THE PROBLEMS AND CONCEPTS OF EVOLUTION.

INTRODUCTION.

The following chapters give the results derived from an attempt to analyze the phenomena of evolution, using chrysomelid beetles of the genus *Leptinotarsa* as materials for investigation. In this investigation I am interested only in processes or reactions productive of evolutionary phenomena, or that are of experimentally proven importance in evolution, because they are the *modus operandi* of the evolution of living substance and are universal in action. The chrysomelid beetles and their transmuted products and characters are of interest only to the extent that they have been convenient reagents for use in the investigation.

It is important to understand clearly the general philosophical conceptions from which we interpret nature and which guide our efforts in the prosecution of research, since this will largely determine the logical, philosophical, and experimental methods used in investigation and the hypotheses created. It is sufficient to state my own position; others must decide for themselves to what extent they can interpret the phenomena of organic life upon the same basis.

In the absence of any evidence to the contrary, the physical world, as known to us from our sense perceptions, is all that exists, and the general philosophical postulate from which I must approach any investigation of nature is that of positive materialism, and all activities, from the lowest to the highest, from this postulate are, in the end, capable of statement and explanation upon purely mechanistic physical relations and reactions.

It is our task to interpret and understand ourselves and nature from the operation of known physical agencies. When we have entirely and honestly exhausted all of the possibilities of physical science, when physical science shall have reached its end, if there remain phenomena not capable of statement in terms of the physical factors of the universe, it may then be necessary to search for ultra-physical causes. At present we fall so far short of exhausting the possible applications of physical knowledge to the problems of living bodies that there is no valid excuse for even suggesting the existence of non-physical agents or causes which find expression in living material objects and reactions.

PHYSICAL CONCEPTION OF LIFE.

In the contact zones between the lithosphere, the hydrosphere, and the atmosphere of this planet, marvelously complex integrations of matter occur, presenting activities and reactions that are conspicuously unlike, in their totality, anything else found in nature, but upon analysis there has been found only the action of physical principles in these living bodies, combined in an immensely complex system of interacting factors, collectively presenting end or superficial aspects, which to some seem sharply and finally to differentiate this product of

a narrow contact zone of great stresses from all other natural productions of the planet. This living substance, which we as part thereof seek so diligently to understand, and the question of whose origin has been a dominating factor in human intellectual activities through all time, presents to all—scientist, theologian, or layman—questions of fundamental interest.

As human understanding of this living substance becomes clearer, and as investigation becomes more accurate and analytical, there is increasing proof of the sole operation of purely physical principles in the production of the characters and reactions of this living stuff, and some of us, therefore, hopefully look forward to that time in the future when all organic phenomena are capable of statement in purely physical terms. Only some few more or less superficial processes are capable of formulation in physical terms, while a wide array of organic activities, of form and species, and in man the intellectual capacities—memory, thought, and study of himself and nature—are hardly capable of accurate formulation upon any naturalistic physical basis; that is, more than the description of our reactions in terms of the general reactions of living materials.

Certain properties common to all of this living substance, the evidence of the remains of this substance upon the planet, with increasing complications of the primitive attributes and qualities common to all, have forced upon us belief in some natural means of production of this diversity and increasing complexity, with the present array of living types, of which man appears to us as the most complex.

It is profitless to debate whether there is in living things any agency or principle above and apart from the rest of the physical universe, whether there be a vitalistic content, entelechy, or soul; any and all of these terms are but designations of a collective unknown, unanalyzed series of phenomena, whose aid is invoked in a causative sense in the effort to present a mentally complete picture of natural causation. The use of the unknown and unanalyzed, as a cause, in the efforts of modern vitalists is not only unwarranted, but is distinctly a retrogressive tendency, much of which is due to the influence of Kantian philosophy. There is no denying the fact that the unknown with regard to the phenomena of life exceeds the known, but at present that which is organized knowledge is vastly increased over that of a century ago, and in this increase understanding has at all times and in all directions been solely along the line of the application of physical principles to the solution of the problems of living substance, so that for the present we are justified in the belief that it is the only safe point of departure in the effort to solve these riddles of living matter.

Further justification for this attitude comes from the fact that not a single advance has ever come from the vitalistic point of view, and while a vitalistic conception or principle may help in certain metaphysical ramblings, or save some frail intellect from despair at the incompleteness of knowledge as to the causation of the phenomena of life, it serves no other end. At no time have any conceptions of this kind given the least aid in investigation or in the attainment of actual results in the solution of our problems. At all points the physical conception of life and its evolution, at present fragmentary, is nevertheless the only conception that has been of any service as a basis for the effort to understand these phenomena, and finally to comprehend what our understanding of these means in terms of cause and effect.

NATURE OF THE INDIVIDUAL.

At present the most important question confronting us, aside from the determination of what life is, is probably the question as to the nature of the individual, which is in nature the unit of action in the attainment of the evolution and distribution of living species.

Two concepts seem to dominate the present situation: (1) That the individual is a mosaic, composed of lesser entities, unit-characters, biophores, or similarly conceived ultimate vital units, each the carrier of some one or more characters of the organism; (2) that the individual is an indivisible entity, or the unit of existence. Between these views there has been in the past no compromise; either one or the other, in the opinion of the adherents of the special concept, must be true, and in the last decade the data derived from the neo-Mendelian investigations has, in the opinion of many, strengthened the mosaic conception.

If the factors and determiners discovered in these Mendelian investigations are thought of as carriers in the Weismannian sense, the evidence obtained by the workers with this principle can be construed to support this mosaic conception. The ideas of the nature of these gametic agents are, however, changing, and the most active and progressive neo-Mendelians no longer regard them as ultimate units, the carriers of characters, but as agents whose presence or absence within or without the organic system is productive of specific end-results or directions of reaction in the general operations of the mass. In this sense the individual and the line of descent must be conceived of in terms of associated gametic and environic factors of composition that must be present and in specific relations for the purpose of maintaining the integrity of the individual in its characters and in descent. In other words, the individual is a compound of physical agents—factors, determiners, inhibitors, accelerators, and so on—not one of which is anything more than some non-living mass or some physical relation within or without the mass, which by its presence decides the course and extent of some one or more resulting reactions and the products thereof in the development of the individual.

In non-living materials there is present in all substances and reactions these agents or factors of operation that are productive of the end-result or the specific compound or substance. Zinc, sulphur, and oxygen are quite unlike when isolated, and all are different from water; nevertheless, when combined under the proper conditions of the medium to permit of certain reactions between them, there results crystalline zinc sulphate, a substance with *specific qualities, attributes,* and *conditions of being,* no one of which was brought in by any of the components, but which are the products of the reaction of the component constituting agents of the specific mass. The mass is a unit, an individualized entity, whose integrity and totality of characters and capacity of reaction is entirely dependent upon the retention in this mass of the position, proportions, and relations of the agents that entered into its production in the first instance, and change in any of these results is a change in the composition of the mass, its characteristics, and in its reaction capacity.

In organisms there is no *a priori* reason why exactly the same conception is not true, both with regard to constitution and also with respect to its capacities for changed reaction. The results of the neo-Mendelian investigations have shown an abundance of instances in which the demonstrated presence or absence

of an agent was definitely productive of changed aspect of the mass, and also of its capacity to react differently when it was combined with some unlike mate in breeding. It is true that in the organic there is greater complexity of composition and reactions present than are found in the simple non-living example; nevertheless, there is no evident reason to suppose that there is any difference in principle of action in them—only in detail and complexity thereof.

There can be no doubt of the existence of these gametic agents that are productive of the exact end-results obtained from the operations of the neo-Mendelian investigators, and while the nature of some of the agents productive of the characteristic in the soma in color are approaching a solution, there is at present entire lack of information as far as the gametic agent is concerned. These somatic agents are, however, entirely physical in their nature, and are undoubtedly the product of antecedent physical agents in the gametes.

These gametic agents which can be so accurately manipulated in the operations of the neo-Mendelian type, giving exactness of result within expectancy of error that is predictable, show that a real start has been made in the problem of solving the nature of the individual and its characters, and provide a valuable means of attack upon some of the evolution problems.

It has often been suggested that only the unimportant and superficial of the organism's characters are found to react according to the factorial principles, and that the "essentially specific" characters do not do so. This is not true, and in the pages which follow I shall be able to show that in some organisms at least many characters, important and unimportant, structural and also physiological, react in entire accord with these factorial principles of constitution and reaction. It is probable, therefore, that final analysis of the composition of any individual species will resolve it into an array of agents that are productive of its total sum of characters and activities, and it is not too much to expect that in time it will be possible to dissect the composition of any race and profoundly alter its character by the removal, introduction, or alteration experimentally of one or more agents or groups thereof, and in several instances in these reports it is shown how this has been actually accomplished by divers means; but in all instances there have been at the basis of the operation either gametic or environic agents, specific and exact in operation.

The individual is an entity to the extent that it represents in its characteristics the summation of the total products of the activities of the genetic and environic agents and their interaction during the development and life of the individual, and this is in no wise different from the specific non-living entity. Each is the product of its productive factors, and in both there are always only physical substances, relations in the system, the product of directive conditions in the medium at the time of reaction and production of the combination. The agents, either internal in the mass or external in the medium, are not the carriers of anything or of any character of the end-product. There can be, therefore, no unit characters in the meaning of De Vries, any more than there are those carried by the pangenes or other ultimate units of other writers; instead, characters are entirely reaction products in the organism, precisely as in non-living masses.

BASIS OF CHARACTERS IN NON-LIVING AND LIVING SUBSTANCES.

Inasmuch as the material elements composing both organic and inorganic matter are the same and exist in both in arrangements of molecules, atoms, or lesser masses, it is the arrangements and reactions of these that must be the basis of investigation and interpretation of organic activities.

In the physical world any mass has certain properties that are properties of the whole, the product of the immense number of interactions between the component molecules. These properties of the whole express themselves in characters, weight, forms of crystallization, ductility, and so on, yet no physicist has ever thought of removing these from the mass and setting each on the pedestal of a representative particle, or of considering it a lesser entity which helps like the bricks in a wall to build up the structure, but they are considered as being what all of our experiences show them to be, namely, properties of the whole, which may be temporarily or permanently altered, but can not be removed as an entity from the mass. This is the condition in the inorganic, and what possible reason is there for supposing that any different condition exists in the organic?

I am not at all concerned with the distinctness with which characters may or may not be present in organisms; these exist and can be experimentally tested and modified, and the same condition exists in the behavior of matter in the domain of physics and chemistry, so that the logical result of the representative particle-unit character conception is the marked admission that the known facts of physics and chemistry are of themselves unable to serve as a basis for the interpretation of the phenomena of living things. I can not form a conception of anything existing in the universe which is not physical in first and last analyses, and although with present knowledge there are difficulties, each added bit of information helps us to understand nature. With this outlook, one can not hope to attain ultimate knowledge of the ordering of living material in nature; there may always be an uninterpreted residue of observed conditions. This, however, is constantly diminishing, and behind we leave the phenomena of nature interpreted in the store of human knowledge as the results of our collective experience. Each one may have his particular opinion, which may be for him the correct one; which of the numerous concepts is correct, however, time alone will determine, and, we may rest assured, will determine correctly.

RELATION OF EXTERNAL AND INTERNAL FACTORS.

It is undeniable that in organisms there are internal factors which condition and govern the production and manifestation of the bodily qualities, and it is equally certain that there are factors external to the organism in the medium in which it lives, and as far as there is any evidence, this has always been the condition in living matter. It is frequently maintained that in the evolution of organisms the factors of importance are entirely internal and all that the external conditions do is to determine which individuals shall live. Others hold that all of the important determining factors in evolution are external and that the living material is a sort of plastic, homogeneous material to be molded by the conditions of existence.

In the domain of physical science it is recognized that any state or condition in any substance or body is the product of two groups of factors—one resident

in the substance itself, the other in the medium surrounding it—and the interaction of these two sets of factors determines the particular qualities, conditions, and attributes presented. In organisms there are both internal and external factors, and the *a priori* view is that they have the same relation in organic matter as in inorganic, and if they do not have this relation it is something to be proved and not held as an *a priori* dogma.

CATEGORIES OF ORGANIC CHARACTERISTICS.

Present considerations of the characteristics of organisms designating them all "characters," without in any respect attempting to comprehend what the nature and cause of the character is, and how modifications may be the product of diverse causes, have lead to much confusion.

In physical terms natural specific things have three grades of characteristics:

Specific Properties or Qualities—Belonging to substances, and not capable of change without change in the nature of the substance except by alteration of the factors of composition; *i. e.,* color, hardness, specific gravity, configuration of the system (crystallization).

Attributes.—Characters distinguishing bodies of the same kind, as size, weight, volume.

Conditions.—States of being or activity, as temperature, motion, etc., that can be changed or removed without altering the attributes or specific properties.

In organisms there is precisely the same series of categories of characters present, which must be considered in the investigation of the evolution phenomena, and care taken that the three categories of characters are not confused in investigation.

SPECIFIC PROPERTIES OR QUALITIES.

Specific properties or qualities are those characters which can not be changed without altering the identity of the substance, and in the non-living this is always accomplished in one of three ways, either by replacement within the system of some factor that is productive of specific end-results, because of its presence, by another similar but different factor which is capable of occupying in the system the position vacated by the displaced factor—a simple metathetic change; or change is produced by the loss of a factor, so that the character is no longer produced; and lastly, changes are produced by the production of new, or changes in present, factors, thereby productive of new or transmuted characters in the substance. This is precisely what happens in the non-living as in the living, and I shall be able to show how in the materials that I have used there have been produced in experiment permanent changes in the specific properties of these organisms by these three main methods of change and by combinations of them. In these modifications three general mechanisms of change can be distinguished:

(1) *Combinations,* or the union into one of two or more factors or factorial groups, producing in the end a combination product of the characters present at the beginning.

(2) *Decomposition* or *germinal disintegration,* the breaking up of a substance or form in which there is loss of one or more of its factors and consequently of one or more characters of the original, producing "varieties" of the original.

(3) *Complex redistributions,* of which two grades have been present in my materials: (a) Double decomposition: Metathesis (the Mendelian reaction), in which there is in the reaction incident to the reproductive process, decomposition of each of the gametic systems present in respect to essentially equivalent and mutually replaceable groups, which then interchange places in the gametic systems of the two gametically different races present in the reaction. (b) Internal rearrangements, in which there is dissociation of the agents present in the gametic system, with rearrangements of them into new relationships, with the end-result that there appears in the resulting individual product either new or changed aspects of the character or characters.

ATTRIBUTES.

Attributes, which are characters distinguishing bodies as size, stature, weight, and the many other characters that serve to distinguish one individual mass from another, or one individual organism from another of the same species, are in the main those characters in the organism that have been in the past chosen for the operations of the "selection process," and for the diverse attempts to alter "quantitatively" the nature of the individual. The differences that fall in the category of "fluctuating variations" belong properly to this class of characteristics, and in the non-living as in the living are in their manifestation much influenced by the conditions of the medium.

In organisms there seems to be a basis of permanent differences in the capacity of these attributes to be manifested. Thus, in stature or in weight (beans) there are values in the population that seem to be limits beyond which change is not possible, and also in some instances values that may be maintained in pure lines without change. What this signifies is difficult to decide, but the investigations of Johannsen, Jennings, and others have at least made a decided step in the solution of the problem. No doubt in final analysis it, too, is the product of the relations of the factors that are productive of the specific properties of the individual, and may be due to many causes in so complex a system as organisms are. In some of the later chapters detailed experiments in this direction show how, in some instances at least, the conditions present are to be explained. It is not to be expected, however, that upon the basis of this conception any alteration of the nature of the substance can be produced by the accentuation of these attributes, because there is no point of entry for any operation that could possibly be productive of any type of change in the specific properties, and because throughout the factors productive of them are maintained in the system in their original relations, and no means is present in the attempt to alter the attributes, whereby a change in the relations or in the factors that are present could result. This is, in my experiments, the reason for the failure of "fluctuations" to carry alteration beyond a certain limit by any means. It is not due to the inefficiency of "fluctuations" and the efficiency of "mutations," but to the fact that in the "fluctuations" only the differences in attributes have been concerned, and in the operations based thereon there has been no method present that was able in any way to change the nature or relations of the specific properties present.

CONDITIONS.

Characters that may be described as conditions in the mass are present as completely in organisms as they are in non-living substance, and in organisms these characters concern many of the reactions and behaviors that are present and that are so easily modifiable. These in the non-living are the product of the nature of the substance as constituted by its factors productive of the specific properties and the conditions of the medium, of which many common examples will at once occur to the reader. In organisms many of the tropic activities and "animal-behavior" characters are of this kind. They may be held in one condition by retention of the same condition in the mass and one set of conditions in the medium, or at any time altered, without in any manner changing the nature of the substance itself, and of these changes I have presented several examples in later portions of these reports. The characters in organisms that are of the nature of conditions are in many instances of great importance in the economy of the organism, and serve immensely vital rôles in the processes of conservation and equilibration as well as in the phenomena of migration, distribution, and apparent composition.

It will, I believe, be at once perceived that the three categories of characters recognized are not only present in organisms as they are also present in the non-living, but that this recognition of the nature of the different categories present in organisms will aid in the further investigation of many problems. It will be possible from this basis to clearly differentiate and formulate the problems of experimental investigation, and in some directions there appears at once a clearer understanding of many problems and results.

The chief advantage gained is the conception of the categories of the characteristics of natural things, living and non-living, from one point of view, and in this there must be at the least an element of permanent truth, since both are the products of the same elements and of the same general types of physical and chemical action.

The literature upon the evolution problems has dealt and must properly deal with the characteristics of organisms, but unfortunately there is an ever-present "biological aspect" to these writings and failure to look at the problems of evolution from purely physical aspects in the majority of instances. Many harmful metaphysical concepts and methods of expression have gained entrance to the evolution literature; nevertheless I believe evolution problems should be conceived of in a purely mechanistic physical sense, devoid of utilities, purpose, motive, or "forces of evolution."

EVOLUTION AND TRANSMUTATION.

That there are in nature different kinds of living substance, each constant in form and continued in endless series of generations as far as our observations go; that there has existed in the past an innumerable array of these specific kinds of substance and that the history of living nature on this planet, as discovered, shows changes of the characters and in the historical sequence of these forms, are undeniable facts. For the most part the facts of the history of life upon this planet are isolated, but suggest to us nevertheless continuity of change in the past, in conformity with some natural principle of causation, and hence there have arisen diverse hypotheses of the causation of this diversity and the sequence of events in the different phyla of the living world.

PROBLEMS OF THE PHYLUM.

Regardless of hypotheses of the causes of the conditions found in nature, two striking facts are shown in all of the different phyla that are known, namely, the fixity of a type of organization that is constant throughout the phylum, and the fact that there occur changes in the different minor features of the phylum, which are responsible for the production of the diversity of specific kinds of living bodies, past and present. Present historical information gives little satisfaction as to the origin of these huge lines of organic form that persist through the history of the planet for inconceivable periods of time, and while interesting hypothetical pictures of the relations and origin of these may be formed, perhaps with some degree of certainty, the fact remains that there is no actual information as to the method or point of origin of any of these major groups of living substance.

Within any of these lines—phyla—the information increases in amount and certainty as our point of observation approaches the present, and in the living things of to-day we see, and in experiment produce, changes in the characteristics of some of the members of different phyla. Methods of transmutation of the qualities, attributes, and conditions of the phyla are thus either proven in experiment or with different degrees of certainty tied to operations in nature, and the whole is then used in the effort to project present experiences into the past to interpret and explain the production of diversity, or the evolution within the phylum through its history as paleontology reveals it to us.

In each of these histories of the phyla, and collectively true of them all, is the fact that their history, as revealed in their remains, shows through time a sequence of modifications, at the start simple organizations, little differentiated from the phyletic type, with subsequent changes resulting in the later members in complicated or specialized arrangements of the originally simple phyletic characters. More rarely in restricted groups, or especially in some portions of the phylum where it is possible to observe in some detail the history of a large group, the history is one of progressive increase in complexity to a maximum of diversity of species and structures, followed by an old-age simplification of the structures and decrease of the number of different types, ending perhaps in final extinction of the series, in which the last members approach in simplified form somewhat the conditions of the series in its younger stages.

There can be no doubt that precise experiment is the only method of obtaining exact information as to the methods and factors in evolution; nevertheless, it must not, in the enthusiasm of the moment, be lost sight of that the formulation of hypotheses of evolution from these experimental results must keep in mind these undeniable facts of the history of any and all lines of living substance. No doubt it will be a long time before experimental efforts will be able to proceed far in the elucidation of this question, but a start has been made, and promises to replace, with certain knowledge of the relations of cause and effect in transmutation, the plausibilities of the past, and thus enable us to undertake more certainly the interpretation of the causes of the production of the forms and history of the inhabitants of the planet in the past.

Concerning the origin of the phyla and the fixity of type within each at the present time, and the production thereof, there is little but opinion or belief as to the origin and causes. These must be accepted as found, their remote history and origin credited to our ignorance and not to ineffective mentality or methods

of investigation, and hope for further historical information that shall in some measure help to close this gap in our knowledge. Complete information as to the nature of the form that seems so fundamentally a part of each phylum, especially as to its method of production and change, is and must be entirely a matter of experiment; and it is not too much to hope that within no distant time it may be possible with increased knowledge of organic constitutions and skill in experimental operations to attack directly and change permanently the basal system upon which the phylum is built.

Fixity of phyletic type is axiomatic and has long been admitted, even when accompanied by differences in theory as to origin and also in the method of producing diversity within it. From the first to the last all have been impressed with the fact that in the history of any phylum there has been uniformly the progress of the type as a whole from the simple conditions when it appeared first in historical records to later periods or to the end of its existence, and this has produced the idea of a movement in the series based upon the phyletic type, and has found expression in different conceptions as to its causation. From Aristotle to the present it has been difficult to escape the cul-de-sac of an evolutionary principle or force which in the specific phylum determines and in a way drives it through the observed series of conditions discovered in its history. The question why may be asked; it can not be answered at present in any certain terms, and belief in any agency helps only individually in forming a comprehensive and comforting picture of nature. Any theory of evolution to be valid and comprehensive must in some manner provide in naturalistic operations for these phenomena in the history of all phyla of living things. There is no evidence of growth force any more than there is of a principle of perfectibility, or evolutionary force, or bathmogenesis, or orthogenesis, in the production of the results observed.

Any naturalistic cause of the history of progressive specialization observed in phyla must at the same time provide for the evident simplification of the members of the series in the later stages of its history, and the return of them to the more elementary states found at the beginning of the history of the phylum. It may be interesting to compare these to the juvenile, adult, and senescent conditions in the span of a single life, and while there may be similar processes productive of the two, as far as present information is available, there is no evidence that it is so, no matter how earnestly one may believe it.

Thus at the base of our conceptions of evolution in nature there lie universal conditions, beyond doubt entirely naturalistic as to production, but at present known to us only from the broad outlines of their results through past history in different phyla and throughout organized nature as a whole. Most probably the phyla are but the expression of these same underlying causes, and could we arrive at an understanding of the nature of a phyletic form and the operations whereby its composition could be permanently changed through experiment, there would no doubt thus be derived the basis for understanding the fundamental nature of these more proximate questions of the apparent movement and the history of any phylum, and in the rise, culmination, and decline in the complexity of the members of groups as the special series passes through the history of the planet.

The problems of phyla and of larger groups within the phylum are a matter of phylogenetic speculation; and evolution theories have, from Lamarck to the

present time, been mainly concerned with the methods of transmutation of the units of natural species. The principal problem of the student of evolution has been to discover the causes of the production of species, and then to be able to experimentally produce them. This investigation in the past century has given several hypotheses of the mechanism of transmutation.

HYPOTHESES OF SPECIES TRANSMUTATION.

None of the several theories propounded appears to give a satisfactory explanation of the problems of origin of species. The formation of species by the Darwin-Wallace factors has many difficulties to overcome, and its later modifications, wherein transmutation was the result of the accumulation of minute fluctuating variations conditioned by the play of mystic entities in the germplasm, involves a degree of credulity not common among scientific workers outside of biologists of the neo-Darwinian school. Others have tried to reestablish the principles of Lamarck that the stress of the conditions under which the animals live induces modifications that are transmitted to subsequent generations. Still others, through inability to substantiate the fundamental idea of the Larmackian theory, and unable to make progress with neo-Darwinism as a working hypothesis, have conceived of evolution as due to a jumping process, and sought for data to prove that new characters or modifications of old ones arose directly through sports, and that these become the progenitors of race and species.

Not satisfied with any of the preceding hypotheses, the attempt has been made to find an efficient cause for the origin of species in geographical and physiological isolation, but especially that geographical isolation which comes from the segregation of the different portions of the same organized form in unlike habitats, and hence in different environmental surroundings; while in another line of effort, orthogenesis, one finds, in the writings of the authors supporting the hypotheses, evidences of the old Aristotelian concept of an impelling principle or of a goal with a fixed line of development in the evolution of organisms. With this array of hypotheses it is no wonder that the layman is confused and inclined to think that perhaps after all evolution may not be as universal a truth as some of its advocates have claimed it to be, and even the biologist often wonders what the outcome of this situation will be.

The oldest of these hypotheses of species formation—that of Lamarck—was formulated when many things now common knowledge were unknown. Lamarck in his time knew something of the direct effect of external conditions in inducing changes in organisms, especially in plants when they were transplanted into the botanical gardens of Europe from exotic locations, and these direct effects of the transplantation were noted, and this seems to have furnished Lamarck with a possible natural cause of the formation of species. Lamarck recognized that unless this modification was transmitted to subsequent generations no evolution could result—which was certainly correct—and hence the idea of the transmission of these through the reproductive process. It is true that Lamarck was never clear as to how these characters were transmitted nor how they could be transmitted, excepting that transmission from generation to generation was in some way associated with the reproductive process and was necessary for the transmutation of species in evolution.

NEO-LAMARCKIANS.

The neo-Lamarckians, although boldly maintaining the truth of Lamarck's fundamental proposition of the effect of the conditions of the medium, seem to have been most uncritical in the use of the cardinal principle of their master, and at the present time there is no evidence that the neo-Lamarckians' hypothesis is true, and it is necessary to suspend judgment as to whether their principle of the transmission of variations from the soma to the germ ever occurs.

The essential principle in Lamarck's work, however, that differences in the medium, and especially those resulting from change in habitat, are effective factors in evolution, certainly must receive most careful experimental investigation, and it matters little whether the effect of the conditions of the medium are direct upon the germ or indirectly through the soma to the germ. The principle is now shown to be true that changing conditions in the medium are efficient factors in the evolution process.

Although forced now to admit the truth of Lamarck's essential principle of the effectiveness of factors in the medium, we do not commit ourselves in any way to the hypothesis of the neo-Lamarckians, nor is it possible to do more than recognize that if their principle is effective in evolution, further consideration and use of it can only be the product of exact experimental investigation.

The chief principle of Lamarck, however, finds a permanent place in the hypothesis of transmutation by means of natural selection as enunciated by Darwin, which was a far broader conception of organic evolution, wherein he made great use of the effect of environment so emphasized by Lamarck. Darwin forcefully and frequently insists that the agencies most productive of the differences in any population of organisms, which become the basis of the selective process whereby species or changes are produced, are in considerable part the direct product of the action of the conditions in the medium upon the organism, and especially upon the reproductive process, and probably upon the reproductive elements.

DARWINISM.

The hypothesis enunciated by Darwin has proven far from satisfactory when applied generally in the attempt to solve the riddle of evolution in plants and animals. Darwin recognized the Malthusian principle that every species tends to increase at a rapid rate and to produce a greater number of individuals than are capable of survival, and that of these 98 per cent in the main die before reaching maturity, only 2 per cent surviving to reproduce the next generation. It is axiomatic that there is this excess of reproduction over survival, and the whole of Darwin's hypothesis of transmutation hinges upon the single point of *whether those individuals which persisted and reached maturity are those which possess variations of a nature such that they are thereby made more efficient than their fellows, and hence better able to successfully compete in the "struggle for existence," or whether the survivors are only those individuals whose chance position, when the accidents of life happen, save them from extinction, and eliminate their less fortunately placed companions.* If it is true that the surviving minority are the possessors of conditions making them more fit to meet the struggle for existence, then Darwin's hypothesis has a considerable degree of probability of truth, but *it distinctly has not been shown that the surviving*

individuals are the fortunate possessors of these advantageous modifications. Because individuals do survive, it is the custom to assume on the basis of Darwin's hypothesis that they are better able to meet the conditions of life than those which were eliminated, but an accurate analysis and measurement of this problem is utterly lacking, nor can it be attacked, excepting through a long-continued, painstaking analytical investigation upon favorable materials in the laboratory and in nature.

If it proved to be true on adequate investigation that the elimination in natural populations is entirely a matter, or even largely a matter, of the chance position of the individuals when the accidents of life occur, and that the survivors are on the average no better provided with equipment for life than those which are eliminated, then Darwin's hypothesis as a means of transmutation and species formation in nature must be abandoned, or at the least assigned a minor position as a productive cause of diversity in nature.

It is not worth while at this place to enter into a lengthy discussion of Darwin's hypothesis. There has been too much discussion and too little investigation; but one aspect needs attention at this place, namely, the idea advanced by many, perhaps most vividly insisted upon by De Vries, that "natural selection" acts solely as a sieve which allows certain individuals to persist, while others are eliminated thereby. This "sieve-like action," however, seems to me to be one quite different from that which Darwin conceived of in his hypothesis. There is unquestionably in nature an action of the conditions of the environment which in the main eliminates extremes in any population, and further, under adverse conditions it is the mediocre in the race which has the best chance of survival. This seems to be a fairly well substantiated generalization, as far as evidence at the present moment goes, and in my own work I have frequently found that this principle was actively operating when populations were subjected to hostile conditions. This action would have a tendency of conservation, to hold the population in a stable condition, and distinctly would not have the action which natural selection was supposed to possess by Darwin—one of producing divergence. It is possible that two operations have been confused, and that there should be recognized the possibility of there being in nature a "natural selective process," which might be productive of the results which Darwin supposed to be the basis of the origin of species; on the other hand, there are clearly in nature operations which do exactly the things which De Vries attributes to natural selection. These latter I have often encountered in my work, and in this report I have adopted the name "factors of conservation," meaning thereby an association of agencies within and without the organisms, which, so long as they remain constant, maintain the race in uniformity. These are agencies acting for the retention of specific form, habitat, and distribution, but they are distinctly not formative forces in the sense that they produce anything new. Their operation is the reverse, and hence their name. In portions of these reports I shall show how experiments with these two aspects of the problem operate in some instances, and especially in relation to the two questions which have been raised with regard to Darwin's hypothesis—*whether it is chance favorable constitution or chance favorable position that determines the survival of the individual.*

Through inability to make progress with the neo-Darwinian concepts, many earnest students of the evolution problems, in the latter part of the nineteenth

century, began a new attack upon the problem of species, which was based in the main upon the conception that if species were discontinuous when fully formed, *a priori* there was no reason why they may not have arisen discontinuously at the start. In support of this proposition there was found in the older writings of plant and animal breeders, and especially in Darwin's works, many records of the rise of new domesticated races of organisms based upon the appearance of an ancestral sport. The attack upon the problem was made from quite different points of view, but nevertheless all have arrived at a somewhat similar result, namely, that certain discontinuous changes in the qualities of the organisms were quite as likely to be productive of evolution as the methods of slow accumulation so currently held by the neo-Darwinian school. Bateson's compilation of instances of discontinuous variation and De Vries's experimental investigation of the process which he calls "mutation" in *Oenothera lamarckiana,* the experimental production and observed rise of stable forms by MacDougal, Tower, Kammerer, Morgan, and others, in the main have provided the basis for the hypothesis that effective changes in the qualities of organisms may arise discontinuously in many directions, producing, as De Vries says, something quite new each time.

MUTATION.

The mutation theory of De Vries has raised a number of interesting and important points for consideration, chief of which are his efforts to distinguish between the conditions present in any population, dividing them into sharply separated categories of "fluctuations," which are ineffective in transmutation, and "mutations," which bear the burden of the evolution process.

De Vries's argument is based largely upon the data of biometric study and the more certain showing that in domesticated plants fluctuating variations are not capable of accumulation beyond an amount which was present in the race at the start as the limits of "variation," and further, upon the showing that all of these variations are either plus or minus additions to the character and in no other directions, while mutations are stable, effective, and in many directions. There is no doubt that descriptively De Vries's account is correct, but I am strongly of the opinion that both the account and the definitions are actually incorrect. I have pointed out previously in this introduction that it is necessary to recognize three general classes of characteristics in organisms, and that of these characteristics one, especially, the attributes, which distinguish bodies of the same kind, has been almost exclusively used in this selective accumulation experimentation, and that there is not present in the process any mechanism whereby the qualities of the race may be altered by accumulation of the amount of the attribute present, and this in every instance is the effort which the worker of cumulative selection seems to have made. With the sugar beet it was increased in the amount of sugar. With other forms it is increase in stature or increased or diminished amounts of one thing or the other within the race which serves only to distinguish the individuals of the race, and not the races from some other; so that in all these operations there is present only the means to attempt to accentuate the attributes present, and there is no possible mechanism available for modifying the qualities of the race to which the attributes belong. Whether these attributes vary only in one direction, plus and minus, is largely a matter of definition and the method of investigation employed; and in

a later chapter data will be found dealing with this problem, showing that the statement is true or not true, depending entirely upon the definitions employed and the point of attack from which the investigation is made.

With regard to the mutations of De Vries, I am of the opinion that De Vries has described a specific operation, which is not the same as the formation of sports and saltations described by other writers, and I would distinguish, therefore, a process of mutating as distinguished by De Vries and shown by him to be present in *Oenothera lamarckiana,* and a saltation process in which there is a single sporadic production of one or more divergent individuals as the result of the single application of the productive force to the parental strain. Both of these types of the production of new conditions in the qualities of organisms are experimentally confirmed in these reports, in addition to those already existing in the literature elsewhere, and should be recognized as distinct as to causation. To these a third process must be added, which also produces discontinuous changes either repeatedly in the same strain or rarely as an apparent sport. Morgan's experience with *germinal disintegration* in *Drosophila* and my experience with this same process in my materials leaves no doubt of the existence of this third category as a general type of operation, the products of which are all now collectively characterized as "mutations."

The three processes are alike only in the sense that they produce at one operation new aspects in the organism which are discontinuous with the parent stock. In mutation there is a process operating in which there is repeatedly produced in succeeding generations one or more new types which appear again and again through a series of generations. I have shown in some experiments how such a race can be produced experimentally and give products which are in every way in animals the duplicate of those described by De Vries in *Oenothera lamarckiana,* and hence I think it necessary to recognize a "mutating process" as distinct from the others which may also give products of quite diverse natures. The sporting process seems uniformly to give but a single result, following the application of the productive cause, and there is thus far nowhere in any of my experiments, or in the modifications produced by MacDougal, Kammerer, or Woltereck, any indication of repeated or of subsequent production from the race of the new types. In many of my examples the change is distinctly that of adding a character to the organism, in others it is the displacement of the character, and so no doubt the sporting process works both ways, either to add or subtract characters to or from the race.

In germinal disintegration there is present an operation in which characters are removed from the race one after the other, but which can be put back into the race by proper crossing methods, thus restoring the original; and differing in many respects from the products of mutation or the products of sporting. This germinal disintegration is possibly the process which is largely responsible for the production of large numbers of domesticated varieties, which can be shifted back to the original condition merely by adding the requisite character.

In these three types of change there is concerned solely the action of genetic and environic factors, either through the combination of agents hitherto separated, and hence with the resulting appearance of a new character, or the actual production of new factors, and the removal of agents, all of which show that the entire series of operations resolves itself into the study of the factorial composition and operation of the gametic substance.

These products of mutation, saltation, and germinal disintegration are largely of laboratory origin, and at present there is entire ignorance in the main as to how these products may behave in nature; and it is not known whether they could or would become the progenitors of specific groups. This is not an impossible subject for investigation, and it should receive experimental investigation by the introduction of newly arising groups into localized natural habitats, and their behavior studied through a series of years. In this manner it will be possible to evaluate the different agencies concerned with respect to their efficiency in the production of diversity in nature.

ISOLATION AND SEGREGATION.

Two other hypotheses call for mention at this place, namely, the supposed action of isolation and segregation so ardently advocated by Warming, Wagner, Gulick, Jordan, and others. This hypothesis of species formation by isolation is little more than the plausibilities of geographical distribution and faunistic study and the juxtaposition of possibly unrelated facts observed in nature, with the presentation of them interwoven into an hypothesis of natural causation, in which the fact of their being isolated from one another is conceived of as the efficient motive force. Within this group of writers one finds various causes asserted to be the agency which produces the modification supposed to result from isolation and environmental actions, the various types of isolation elaborated by Gulick, and many others, wherein conceivable but unproven agencies are called into action to explain the conditions found in nature. Habitudinal geographic factors may well be effective agencies in the production of heterogeneity in nature, but these need to be investigated by experimental methods, and I have found in some of the species that I have used that there were degrees of geographical heterogeneity associated to a greater or less extent with different habitat complexes surpassing in refinement that recorded by any worker. All of these differences, however, are entirely the product of distinct gametic factors, which can be determined and experimented with through modern methods. I have likewise attempted preliminary experimentation in nature in the effort to create isolated colonies of species of like gametic constitution, and then to test repeatedly the nature of these species in the effort to determine whether isolation is productive of change, and, if so, to what cause the changes are due. Thus far the results obtained show that isolation alone is not productive of germinal changes, provided that homogeneous materials are used for the experiment and as long as the new conditions do not operate as effective factors in the production of gametic changes by the direct action of the environmental conditions. Isolation unaccompanied by such environmental action never shows, even with the closest inbreeding, any change in characters, and there is no evidence that with gametically uniform stock there is any possibility of such processes resulting in change. When stocks are not uniform or pure, then there have resulted isolated races in these experiments, and this experimental production of them may help in interpreting the conditions in nature, and aid in the solution of the origin of many geographical races.

ORTHOGENESIS.

Lastly, there is the hypothesis of evolution which is characterized "orthogenesis." It is notorious that, since the time of Aristotle, through the Middle Ages, in the recent writings of Nageli, Eimer, Hyatt, Jackson, Whitman, Osborn,

and others, the fact so often recorded in nature, that modifications within the phylum and in different minor divisions thereof, the supposed succession of species through successive geological horizons, or over some terrestrial surface, appear to have the relation, one to another, of a series of steps which seem to have moved from the first observed condition to the later one of greater or less complexity, and the fact that in ontogeny the sequence of stages during development is always uniformly the same, and that this ontogenetic sequence to a greater or less extent parallels that found in phylogenetic series, has led many to postulate a causative agency which maintains organisms in these lines, giving specific end-results. Hence the word "orthogenesis," used descriptively by Haacke, has come to a greater or less extent to designate an unknown but active principle, whose effect is to produce these modifications, one after the other, in an orderly, step-like series. There is no denying the facts of our observation as to the series both in phylogeny, ontogeny, and in the phenomena of distribution, but it is not warranted to personify the unknown agencies which were productive of these conditions and make the unknown an effective cause. A cause there must be, but that there is a force driving organisms through nature, or an evolution-trend moving towards a goal, or a principle of perfectibility is something which the majority of biologists are unable to believe. As a matter of fact, it is highly improbable that the observed conditions described by the word "orthogenesis" are produced by uniform causes at all, and results that might descriptively be called "orthogenetic" might well be produced by quite heterogeneous causes. There is no positive evidence, aside from plausible interpretations based upon the hypothesis, that the conditions, placed one after another in paleontology or in distribution, really are in sequence with one another; nor is it known by exact demonstration in any instance that one arose from the other. The data derived from ontogenesis and the frequent parallelism between ontogenesis and phylogenesis does not signify anything more than the conclusion that like substances react alike under similar conditions, and that the same reactions follow, under similar conditions, the same sequence of events. In physical nature identical substances and identity of capacities under the same conditions produce identical reactions, and show the same array of sequences; and so I should regard both the ontogenetic and phylogenetic series as merely recurrent operations present in essentially the same substance, and they are not to be interpreted as due to a principle driving organisms through a predetermined series of events. The data of orthogenesis are entirely of an observational character, based upon studies in phylogenesis, geographical distribution, and ontogenesis; but there is no evidence that there are productive forces, or a force at all, apart from the other natural operations, and it is possible to find an explanation for these states which are observed in nature that is in harmony with other aspects of the evolution problem.

With this array of hypotheses conflicting in principle in some respects and overlapping in others as to the production of species in nature, one is made to wonder whether after all there may not be a common basis upon which they can all be tested and brought into agreement. Each one has its good points as well as its defects, and if a common basis of operation and investigation can be found it will be possible to test the respective propositions of each of the divergent conceptions.

PROCESSES OF EVOLUTION.

The investigations of the past century have clearly formulated certain definite evolution processes which may be characterized as *ontogeny* or *development, variation* or *heterogeneity, heredity, conservation,* and *equilibration.* These comprise groups of activities which are involved in the production, in determining the survival and final location, of the products of evolution.

HETEROGENEITY.

The process which has so long been characterized "variation" is distinctly not a uniform one, but a collective name for the results of diverse reactions whereby there are produced in natural things different degrees of unlikeness in many directions, or heterogeneity. Because of this absence of unity of action, it is more correct to think of the production of heterogeneity in nature rather than of "variation," because heterogeneity is simply descriptive of the actual results produced by many processes, while variation implies *divergence from the average or mean condition.* This conception of variation reaches its climax in Darwin's work and in the neo-Darwinian hypothesis is unduly accentuated by the biometricians, but it is distinctly not applicable to the problems as understood at the present moment. "Variation" in the physical sense implies simply the finding of a range of values above and below some mean, divergence of results produced by conditions external to the reaction, which cause it to diverge and show error or departure from the mean or true value. Such arrays, common in all physical measurements, are adequately expressed and formulated according to the theory of error and arrangement in various types of frequency curves. On the other hand, it is known at the present moment that the highly miscellaneous differences which are found in any natural population are distinctly not the product in any population of one cause, but of many, and so the diversity existing in any population is better described as heterogeneity.

It is certain that within any natural population there may be produced diversity not only as the result of the effect of the conditions of existence upon the developing organism, but also as the direct result of the composition of the organisms, and these again may interact to produce other added complications in the array which the population presents, and when to this are added all of the possibilities which came from the combination and recombination of the numerous unlike factorial potentials in the gametes and the unknown series of operations which may produce new factors in any generation, it is certain that the problems of diversity in natural populations are among the hardest problems to analyze at the present moment, and I have devoted in this report considerable space to the attempt to analyze some of the problems of heterogeneity as it finds expression in these organisms.

In this respect the conception of the natural diversity of any population again agrees with the diversity present in any non-living group. Their multifarious conditions are the product of numerous unlike causes and not of a single operation, and out of the array present in any population two general types of result seem to follow in the next generation. There seem to be distinctive conditions present in the population which are not repeated in the next, and these are the "fluctuations," according to the Weismannian point of view, which are "somatic" in character and not "germinal," hence their "non-inheritance."

On the other hand, there are characteristics which are repeated in the next and following generations, frequently for long periods of time; these are, therefore, asserted to be "germinal."

We have been too lenient with the Weismannian dogma and the criterion it establishes, that because a character does not appear in the next generation it is not a product of germinal origin; but there is no longer any doubt that there are conditions in the population that are entirely the product of gametic factors which may not appear in successive generations, due solely to the fact that the right combination of factors is not made. A further fault at the present moment is the assumption that because a character is not "inherited" by the progeny it therefore is somatic in character, and while I do not doubt the existence of modifications which are purely somatic in character and are, on the basis of present knowledge, hardly to be expected to be of inheritable kind, nevertheless the backward application of this test is not justified, and I shall be able to show later that there are characters which "do not appear to be inherited," which are purely germinal in origin. Furthermore, it is shown in some of this work that there are conditions appearing in populations which would be characterized by De Vries as fluctuations, and which are not capable of fixation in permanent form and are not inheritable, which are not somatic, but germinal, due to the presence in the gametic system of gametic factors occupying positions or relations such that their action is to produce this irregularity. In other words, they act as impurities in the gametic system in the same way that impurities may act in non-living physical systems.

INHERITANCE.

Inheritance is also simply a collective term descriptive of the reaction of gametic agents and their behavior through a series of generations, and the results which may follow from the combination of specific gametic factors. It is hardly possible at the present moment to distinguish between the phenomena called "variation" and "heredity"; and operations often characterized heredity are distinctly important in the production of heterogeneity in natural populations.

These two groups of phenomena, heterogeneity and heredity, are the basis, of course, upon which evolution works, and present problems are, therefore, of two general kinds: First, by means of the principles which have been developed in the last fifteen years, there must be undertaken an extensive and accurate analysis of the gametic constitution of organisms in terms of their component factors and determining agencies; secondly, with this knowledge gained and with materials of known gametic constitution available, to analyze the phenomena of heterogeneity, under the controlled conditions of laboratory experimentation and also in organisms in nature. In combining these two I have attempted to trace in some degree the significance of heterogeneity in several species of these organisms, and this effort, I believe, gives a clear insight into the real significance of natural diversity and the potentialities present in natural species for the production of divergent groups, and through this means perhaps there is a new method of attack upon the problem of origin of species. Actual evolution operations of course center in and around the processes concerned in the production of heterogeneity and the operations which are termed collectively "heredity." Everything that ever has been or ever shall be in the evolution of

organisms is the product of operations within this domain. They supply to the race the qualities, attributes, and conditions, the modified old, the new characteristics, either in combinations or separate, arranged to produce the specific living kinds or species which must after their formation by the operations mentioned be subjected to the tests of the *factors of conservation,* and lastly to the *processes of equilibration.*

CONSERVATION.

The *factors of conservation* are those natural relations of the internal and external factors of every organic kind whose relations determine at once and for all whether or not the organism can persist. This is perhaps a synonym of natural selection, but natural selection is employed in so many ways to mean an agency productive of diversity, or as an agent which is productive only of elimination, that I prefer the term "conservation" as indicating those operations in which the newly arisen mechanism is tested out and it is proven by test whether it is able or not to survive under the conditions into which it is thrust by the process of origination. This is a complex test and it can only be investigated by exact experimental analysis. No information can come from the routine collecting of statistics and their treatment in biometric fashion, and thus far the only information that I have been able to obtain concerning the operation of the processes comes from experimental test in which it must be proven what the specific eliminating factors are and how they work in the system. I have experimentally tested a number of examples in this investigation, and in every instance it has been found that elimination or failure to pass the test of conservation is entirely due to the factorial composition of the organism, and that a factor of one kind or another by its very presence forces upon the organism either in mode of action, or in some quality, attribute, or condition, relations or methods of reaction such that in some portion of its life the relations between external and internal can no longer be maintained, and the organism is either eliminated by its own death or the race is eliminated by the inability of the organism to reproduce itself under the conditions.

I have thus far failed to find any influence of those selection effects, and in every instance elimination, as far as I have observed it and tested it in these and other organisms, involves little of the "biological content," and is largely, if not entirely, a matter of physical relationship between the factors of composition within the organism and the physical factors of existence without, in the medium. This aspect of the problems of evolution at the present moment needs extensive experimental testing, and must be carried out, in order to obtain the best results, in combination investigations in the laboratory and in nature, and in this investigation I have been fortunate in the nature of my material and with the facilities which the American continents have afforded for various types of experimentation in nature.

EQUILIBRATION.

When an organism has passed, if by its original constitution it succeeds in passing, through the test of conservation, it must then meet an added series of problems in the activities which are characterized *equilibration,* which are those activities whereby the organism, without changing its qualities, so adjusts its attributes and conditions to the composition of its medium that there is the least

possible resistance between it and the conditions under which it must live. This is a specific set of reactions which every species must undergo, and I have seen many interesting examples of it in the introduction of one race from one habitat to another, in which the habitat was not sufficiently different to serve as an inhibition to its existence there, but in which there were sufficient differences in the new habitat to bring about in the introduced race distinct adjustments of the organism, not in its qualities, but in its attributes and conditions, which may become definitely altered and thereby produce a new localized habitudinal race. When an organism has passed through the processes of origination, has been tested as to its ability to survive, and has adjusted itself to the location into which it is placed, it takes its place as a member of the community, and, in as far as anything which it exhibits is concerned, it may have been in that community for hundreds of thousands of generations, when, as a matter of fact, it may have been there less than a half dozen.

This brings us to an important point in all of these operations in evolution, namely, that when there is change it is rapid; that the processes of origin produce transmuted factors in the organism within one or two or at the most a few generations, and that the product appears finished and complete in all its characteristics in a single relatively short series of processes. This may not be universally true, but in any event the changes which I have observed have reached completion in a short time, and the questions of conservation are decided at once. The organism with its system either can or can not survive under the conditions in which it arose, and this in every instance is decided in one or in the most two or three generations, and the operations of equilibration are again rapidly brought to a stable condition, so that in a half dozen or a dozen generations from the beginning of the process of change the organism, so far as appearances are concerned, in its stability and in appearance, might have existed in its habitat for ten thousands of years.

Many of the previous conceptions, especially those of Darwin and neo-Darwinian schools, demand long-continued, faltering operations with irregular, haphazard reactions, when as a matter of fact in observed instances the operations and reactions are as rapid, as specific, and as complete within a short time from their start as are the operations in physical phenomena. This must of necessity be so, because in all these operations there are involved only the physical component agents within and without the organism; its gametic factors and the processes which we analyze and describe, which are nothing but the interaction of these physical substances and relations in terms of physical process; and this is the reason, apparently, why the reactions which have been witnessed in experimental evolution have given so radically different a viewpoint with regard to evolution problems. This brings us to the last point of consideration—the point towards which we have been moving for more than three centuries—namely, the question of an adequate formulation and experimental investigation of a factorial theory of organic evolution.

FACTORIAL THEORY OF EVOLUTION.

It is difficult to decide where the first ideas of a factorial theory of evolution arose, but certainly we can follow the development of it for a long time. This is common knowledge, and everyone knows how Mallet, and Maupertius more than a century ago, formulated factorial concepts of organic constitution and of

evolution. We all know to what use Spencer, Darwin, Weismann, and De Vries have put this conception in the production of their theories, but Darwin, Weismann, and De Vries figured their factorial elements in the gamete as single bodies, the carriers of the characters of the organisms. Each of these bodies, in the terms of these writers, was potentially a small organism. Any such conception eliminates the possibility of investigation from an experimental point of view, and is certainly of an order which can not be formulated in any physical terms. There is no known instance in which a substance is the carrier of a character, and all characters which appear in non-living substances are the reaction products of the combining factors of composition and the conditions of the medium at the time of combination.

Out of the neo-Mendelian investigations there has come the most valuable contribution to biological science in a long time, namely, the exact demonstration that the characteristics of organisms are the product of the action of two or more productive agents, and the Mendelian operations have clearly shown that these factors may be removed one from the other, and that one may be removed and the other left, thereby producing the non-appearance of the character with which the original pair is associated, and that the character may be brought into existence again when the missing one is replaced by the action of crossing. This gives an opportunity to apply this principle further to the analysis of organic constitution and to evolution problems. In this report the principle is applied broadly and in divergent lines, in every respect confirming thus far the broad fundamental principles which are the outgrowth of a long series of antecedent studies. And thus we may reformulate our conceptions of organic constitution and evolution, that the organism itself in its gametic make-up is but the associate combination of an unknown number of non-living factors the interaction of which in many associated relations is necessary for the production of the living substance and its characteristics. Any change in characters which the organisms undergo are of the same order and due to the same causes as are characteristic of changes in non-living substances. The method of change of each of these characteristics is entirely the product of the factors, their presence or absence within the system, and their interaction with the factors within and without; likewise the facts of survival or conservation, of equilibration, and of all the processes present in evolution are directly and solely to be investigated and solved from the standpoint of the genetic factors within the non-genetic factors without the organisms.

I realize that it will be contended that while this may apply perhaps with some truth to the superficial characteristics, it can not apply to those long-time trends of evolution and to the problems of fixity of type within phyla. As a matter of fact the conception does give a naturalistic conception of these portions of our problems. In the non-living world are found combinations of various factors of composition which are most marvelous and tenacious in their association. Silica and oxygen form an association—quartz—which is dissociated with difficulty and one which persists for a long period of time. This substance becomes the basis of many minor operations in producing the various kinds of quartz—silicates and their products—which are produced by minor additions or combinations of the basic combination of SiO_2, and this might perhaps be the equivalent in a rough sense of the general phyletic type of organisms which when analyzed show certain securely combined gametic factors,

and it seems to me that that "phyletic type" is nothing more than a chance association of gametic factors, such that it is disrupted with extreme difficulty, but which has also great capacity for adjustment to meet widely varying conditions of existence, capacity for slight added changes, and nothing more. If this is true, then it is to be expected that at some future time it will be possible to dissociate the various factors productive of a phyletic type and perhaps change the phyletic type experimentally. This may be something which will not be accomplished for a long time, but it is not unthinkable that it may be accomplished at any moment with the methods now available. Likewise from the same basis of factorial composition long trends of evolution which one finds are but the potentialities present in these types for the continued successful addition, reconstruction, and rearrangement within localized or trivial portions of the original factorial composition of the race. This view finds more or less of confirmation in the expressed opinion frequently given by paleontologists that evolution after all consists merely in the specialization or in the reduction of the characters of the original progenitors of the group, and this conception does account for and provide us with an experimental basis from which to investigate the condition characterized as orthogenetic.

In physical operations, given a specific composition and conditions in the medium, a given cause will produce a reaction only in one direction, and this reaction will follow through a definite predetermined series of steps which are entirely due to the relations existing between the factors of composition within the mass and the process of decomposition, rearrangement, and loss of factors, and it is probable that the orthogenetic sequences where they exist at all are due entirely to this cause, which is purely naturalistic and is capable of exact experimental production and investigation.

The factorial conception is certain to prove the most usable working hypothesis available at the present time. No doubt its formulation at the present moment, and our description of the factors and the designation of them, are in crude terms a pragmatic personification, but it is recognized that these are at present only symbolizations; nevertheless there can be no reason to doubt the existence of these factors and the type of their reactions in the gamete, which are characterized as germinal factors, any more than to doubt that there exists in the medium various physical agents which are external factors in evolution operations.

CHAPTER II.

THE MATERIALS, THEIR TAXONOMY AND NATURAL HISTORY.

INTRODUCTION.

The purpose of this chapter is to give as clear and complete an account of the materials and their origin as is possible, so that no matter how taxonomic groupings may change, there may be no uncertainty as to the characters, adult and juvenile, and the actual locations from which my materials came. All figures and descriptions are from the living animals, and are the species with which I worked. The findings in experiment through this report are those for a specific form, from a precise location in nature, and no more. I do not know that the same "species" from another location would act the same in duplicate experiments; in fact, many of my experiences show that they do not, due to diversity of the gametic composition of a phenotypical constant species that is uniform in taxonomic aspect over its area of distribution, but is gametically dissimilar. Until we know more of the geographical distribution of this gametic diversity, records must be for the reactions of specific materials from specific locations, and not be applied broadly to phenotypically uniform species. Gametic uniformity must be proven and not assumed.

METHODS OF OBTAINING MATERIALS.

My materials without exception have been derived directly from nature, from definitely located stations which have been under as constant observation as was possible during the period in which the material was being used in the investigation.

In every instance where any material has been used in these experiments the following routine has been followed: Having determined by preliminary tests that the species was of interest in connection with this investigation, I have then, first, gone personally over the entire known geographical range of the species in question, with the dual object of discovering its actual habitat and the geographic distribution thereof and the total range of variability of the form both as regards its common fluctuations and accidental somatic aberrations, and also to discover if, in any restricted habitats, it had developed isolated local races of any description. Further, I found it helpful to become acquainted with all of the conditions under which any species was living and did live in nature. Two sets of information concerning the species were thus obtained—an extensive knowledge of the range of "variability" of the organism and at the same time data of its conditions of existence. In itself this is no small task; it was further complicated and made difficult by the conditions prevailing in Mexico and Central America, where much of this portion of the work had to be done.

At the same time that these field observations were in progress materials were sent in from the field to the laboratories at Tucson and Chicago, and there

subjected to tests to determine their value for experimental work. Their adaptability to cultural conditions and the rapidity of reproduction were of course vital points to be determined, but the gametic composition of the species was of far more importance. Adaptability to cultural conditions, to a very considerable extent, can be attained with most of the organisms that I have attempted to use, and is thus far, in all instances in my experience, entirely a matter of external conditions.

The question of composition, however, is much more serious, and some otherwise admirable materials have had to be discarded on account of the practical difficulties encountered in the reduction of the material to a gametically homogeneous state. The chief difficulty has been the existence in the materials of diverse minor strains which could be isolated by one process or another and then maintained as "pure cultures." Species from nature that had no habitudinal races or modifications of any kind, that were of low somatic variability in response to the changing conditions of the medium, nutrition, and kindred environmental factors, and free from minor strains, biotypes, or other subdivisions, have been the type of materials which I have sought for use.

With materials of this character, and with a knowledge of the total range of the "variation" over its area of distribution, and of the conditions under which it lives in this range, I believe that I have a proper basis for an investigation of evolution, experimentally. The actual materials used in the investigation have in all instances been taken from some accessible location, not likely to be disturbed by agricultural or other economic operations, and during the course of the experiments, or at least during the crucial portion thereof, the exact locality from which the material came has been visited at least twice in each year at a time when the population could be seen in numbers, and at that time a census was made to determine the presence in the population of any divergent or modified traits or characters that were not found in the first general census of the species. *This knowledge of the status of the materials in nature at the point of origin of the experimental material is of the greatest importance, because if any modifications are produced in experiment, and if these are new or striking it is then necessary to know positively whether the new character was produced in experiment alone.*

I have been exacting upon this requirement in the character of my materials for the reason that the bane of experimental evolution at the present time lies in the unfortunate fact that all too often, as in De Vries's œnotheras they were "mutating" when he found them, and everyone is now at liberty to "interpret" the results of De Vries as his own biological orthodoxy dictates. The rigid conditions demanded in physical science should be for the biologist the model of operation, a most rigorous study of the materials in nature, purification and standardization, and then experimental operations with the proper setting to give exact answers to specific problems.

CHARACTER AND SOURCE OF MATERIALS.

THE LINEATA GROUP OF LEPTINOTARSAS.

The animals which have furnished the materials upon which this report is based are a homogeneous group of species—the *lineata* group—of the genus *Leptinotarsa*. These organisms are confined almost entirely to North America,

do not occur in the Antilles, and, as far as is known, are distributed from the northeast corner of South America northward through Central America and into North America as far as southern Canada. It has been difficult to determine what names should be applied to many of the forms found in nature, due to the fact that for most of the species the material existing in museums is meager, often inferior in quality and data, and, moreover, the taxonomic descriptions are based upon dead and often upon only a few badly preserved specimens. Because of post-mortem changes in color, systematic descriptions, especially of the colors, are in the main incorrect for the living animal, and since students of the physiology of evolution are only interested in living organisms, I have given for all species used in this report diagnoses from the living material.

These species are specific kinds of living substances, each with its specific qualities, which, in each, are genetically reproduced true to type, generation after generation, in experimental cultures and in nature. To prevent confusion and to make clear the exact nature and source of my materials, I have presented in this report as complete a taxonomic description as is at present possible of each of the different species in the *lineata* group, their juvenile stages, distribution, and ecology, and such discussion of their life-histories, distribution, and other activities as is necessary to an understanding of the work described herein.

The descriptions are, as far as possible, compiled from the original sources, and I have given the original descriptions as printed and supplement each with a description of the living material that has passed through my hands. Many questions concerning the nomenclature have presented difficulties in the effort to arrive at a just settlement of the complicated taxonomic situation. For example, four species (*L. panamensis* n. sp., *L. guatemalensis* n. sp., *L. diversa* n. sp., and *L. undecimlineata* Stål) are distinguished with difficulty as far as museum specimens are concerned, but they are definite, clearly distinguishable organisms when alive, especially when followed through their life-histories, and when crossed their differentiating characters are sharply alternative in behavior. The most conspicuous differences between the four species are found in the larval stages, and in their habits, distribution, and ecological relationships. Stål (1858) and in 1862 in his Monographie des Chrysomèlides de L'Amérique, described *L. undecimlineata,* and as far as his description is concerned it would apply fairly well to the imagines of either of the forms, and as far as the distribution given by him is concerned it would also apply to any one of the four found, but the one which I have designated *L. undecimlineata* is more probably the one which Stål had, and it is certainly the one which has in later years most often been so designated. Much later, Dugés described the larvæ and life-history of a form from Guanajuato, Mexico, which he believed to be *L. undecimlineata* Stål, but his description is of the juvenile stages of the form that is here recorded as *L. diversa.* Of the two forms, that which has the wider distribution as given by Stål I have called *L. undecimlineata,* and this is in accord with the identifications given to many museum specimens. In the Baily Collection in the British Museum there is a specimen labeled, "Coll. Baily," named by Stål "*L. 11 lineata,*" "Type Stål 194"; "Mexico," that is the same form that I have called *L. 11 lineata* so that there can be little doubt but that the determination is correct. The other I have called *L. diversa,* although many specimens of this species exist in collections and are labeled

L. undecimlineata Stål. *L. panamensis* nov. sp. from Panama, Costa Rica, and the Isthmus of Darien in Colombia, and *L. guatemalensis* n. sp. are also quite as distinct as the other two. The four are closely related and probably represent species that have been differentiated from the same original stock, and which are now limited to separate geographic areas. In that I do not know of intergrades between them, and that their chief differentials are sharply alternative in crosses, I have retained them as species and have not classed them as geographical varieties.

The relations existing between the species *L. multitæniata* Stål, *L. oblongata* nov. sp., *L. melanothorax* Stål, *L. multilineata* Stål, and *L. decemlineata* Say are confusing. As far as I can discover, *L. melanothorax* does not, at any locality in nature, sustain itself as an independent type, but always appears periodically, sometimes rather irregularly, as a recurrent sport from *L. multitæniata* Stål and its geographic varieties. Upon its appearance as a sport in the population, it crosses back with the parent species, within which it is at once submerged. However, when isolated in a culture, it breeds true indefinitely as a specific kind of living substance, and behaves in the same way that organisms which are called elementary species and mutations do. The question as to what *L. melanothorax* Stål is depends upon one's point of view and one's definition of a "species." I have classed it as a "recurrent mutant."

Stål's account of *L. multitæniata* and *L. multilineata* leaves one in doubt, as far as the descriptions are concerned, as to what use should be made of the two names in describing the series of forms which live on the southern half of the high plateau of Mexico at the present time. Throughout the entire southern end of the plateau, southward into the Rio Balsas Valley as far as Matamoras de Izuca, eastward to the Plains of Apam, and northward into the upper tributaries of the Rio Amacusac, Rio Coetzala, and Rio Atoyac, is distributed a variable organism, which is unquestionably *L. multitæniata* Stål. Throughout a considerable portion of the Oaxaca-Guerrero highlands, the Balsas Valley, and up the slope of the Mexican Plateau as far as the foot of the escarpment, is another species closely related to the former, but different in form, distribution, in juvenile characters, as well as in life-history. In some respects this species agrees with *L. multilineata* Stål; but Stål considers his *L. multilineata* and *L. decemlineata* Say to be one and the same species, and *L. decemlineata* Say is not known to me in Mexico. Stål's species, *multilineata,* is, I believe, simply a "form biotype" of *L. multitæniata* Stål that is fairly common in some localities; at least, a biotype of *L. multitæniata* Stål is found which in all respects is like *L. multilineata* Stål and therefore I have used his name for it. The form is radically different from *L. decemlineata* Say and is distinguished by certain gametic factors for body proportions from *L. multitæniata* Stål and its other varieties. *L. decemlineata* Say, as recognized by Rogers and all the later American writers, is clearly distinct from the Mexican types, although in museum specimens the several species might easily be confused, especially when badly preserved. The living animals, however, when followed through the life-history, leave not the slightest doubt as to the existence of the specific entities.

Thorough searching of the European museums, especially the British Museum, has shown interesting points that have materially helped in the solution of this confused taxonomy. In the Baily collection in the British Museum there is a

specimen labeled, "*multilineata* Stål," with the pin labels, "named by Stål," "Type Stål 176," which is evidently an immature specimen, the elytral stripes being brown instead of black. It has, however, the characteristic dark legs and ventral surface and the narrowed pronotum, with the same type of pattern, and in all respects it agrees with the form that I have called "biotype" *multilineata* Stål, and I think that there is little doubt that *multilineata* Stål is a biotypic form of *multitæniata* Stål, and that *oblongata* is a distinct species.

There are in the British Museum five specimens, all alike, that are clearly the same as the *oblongata* recognized here, and that are labeled *multilineata*. Two are from "Oajaca, Mexico," one from the "Baily coll." "Mex.," one with no locality, and one that is marked "*multilineata*, agrees well with description." All of these are, however, clearly *oblongata* and not the biotypic form of *multitæniata* Stål, here designated as *multilineata*.

The species *decemlineata* Say and *multitæniata* Stål, are in all respects so distinct that there is no need for confusing them, excepting in very badly preserved museum materials. There is not the least doubt in my opinion that Stål's species *multilineata* is a biotype of his distinct, widely distributed species *multitæniata;* that *oblongata* is a distinct species, as is *decemlineata* Say, although the three are badly confused in all collections and in the taxonomic literature of this genus, and this is especially true of Jacoby's determinations of these species, as shown by an examination of his materials deposited in the British Museum.

Similar difficulties are encountered in the case of *L. defecta* Stål. The type of organism which has been designated *L. defecta* Stål by most of the American writers, and so labeled in collections, is a close relative of *L. juncta* Guer., has been described as *L. texana* by Schaffner, and apparently represents a modification of *L. juncta* Guer., which has arisen on the semiarid plains of the Rio Grande Basin and nearby areas. Of *L. defecta* Stål I have had live materials from Brownsville, Texas, and it is clearly closely allied to *L. juncta* Guer., and its geographic variety *texana* Sch. is apparently rare and may be only a rare sport from *texana* Sch. At least, I do not know that it anywhere exists as a constant, genetically perpetuated member of the fauna of any locality. Stål's original statement that its habitat is "Mexico, Texas, and Yucatan" gives nothing upon which one could base an opinion, and furthermore, his description and Jacoby's figures are not very illuminating.

The "*defecta*" which I have used in my experiments is not the *L. defecta* Stål, although so labeled in most collections, but is *L. texana* Sch., which is not a variety of *L. decemlineata* as Schaffner supposes, but is related to *L. juncta* Guer. as shown by the character of the elytral punctation.

The *lineata group* represents a homogeneous assemblage of species centering about the lower end of the Mexican Plateau and presents three main trends of evolution, and from present knowledge may be grouped into three divisions as follows:

The species *L. panamensis* n. sp., *L. guatemalensis* n. sp., *L. undecimlineata* Stål, *L. diversa* n. sp. (habitudinal variant *rugosa* n. var.), *L. angustovittata* Jacoby, and *L. signaticollis* (biotype *nigropunctata* Sturm) represent a group entirely confined to the grasslands below frost-line, as shown in plate 3, a tropical savannah group, and all feed upon perennial solanums of the species *S. lanceolatum, S. hegerii, S. chrisotrichum,* and allied species.

The species *L. multitæniata* Stål (habitudinal variant *multitæniata* Stål, recurrent mutant *melanothorax* Stål; saltation *tacubayensis;* biotype *multitæniata* Stål; habitudinal variant *variabilis* nov. var.; habitudinal variant *intermedia* n. var., recurrent mutant *melanothorax* Stål), *L. oblongata* n. sp. (biotypes, (*a*) red, (*b*) orange, (*c*) yellow), *L. rubicunda* n. sp., *L. decemlineata* Say (saltations *pallida* n. var., *defectopunctata* n. var., *minuta* n. var., *tortuosa* n. var., *melanicum* n. var., *rubrivittata* n. var.) represent a group entirely confined to the temperate and cold uplands of Mexico and the plains and prairies of the United States and Canada, as shown in plate 5—typically temperate or upland savannah inhabitants, and all feed upon solanums related to *S. rostratum,* or *S. elæagnifolium* or *S. tuberosum.*

The species *L. juncta* Guer. (habitudinal variant *texana* Sch.), *L. defecta* Stål, and *L. tumamoca* n. sp. represent a group divergent from the two above groups and inhabiting portions of the Atlantic and Mexican Gulf Coastal Plain and Pacific coast desert, as shown in plate 3, feeding upon annual and perennial solanums of diverse kinds.

That I may clearly establish the nature, quality, and source of my material, I have gone fully into the taxonomic study of it, especially in the living animals, their distribution, habits, and ecology, and in this paper I have presented the essential facts known bearing upon these points. It will be necessary to repeat some facts and data already in print, but completeness in the treatment of this portion of the subject is essential at the present time. The diverse series of experiments, to which various members of this group have been, are now being, and will in the future probably be subjected, demands that no effort be spared in ascertaining the uttermost information concerning the condition and relations of the materials in nature.

TAXONOMIC AND GENETIC DIVISIONS.

At the outset of the study of this aspect of the problem I am confronted by the question of what it is that constitutes a species; further, there are the problems of the lesser units or divisions present in some of the species, and of the orientation and terminology to be applied.

In nature there exist only individuals, each with its genetic line of descent, and the aggregation of these genetic lines into groups, and it is this latter aspect of the problem that presents the greatest difficulties. The mere fact that a form breeds true to itself is no criterion and no reason for considering it a species, and were this basis adopted an almost infinite number of species are possible even in one narrow group, depending upon the skill of manipulation and the minuteness of the differentiating characters employed by the observer.

In this investigation I am of necessity forced to consider and use species, and I have from experience come to think of them as organic systems distinguished by a limited array of qualities, which perpetuate themselves in nature, and are separated by a distinct complex of qualities from other related species. Within any such group there are potentialities present in the diverse qualities and their permutation for the isolation, by genetic methods, of a varying number of pure-breeding lines. Also, in some specific groups in one or more areas, the accentuation or suppression of one or more of the qualities marks off the members inhabiting that area into a geographic variety. So, too, there are occasional sports, appearing one at a time and at irregular periods, and also regularly recurring

variations of the same order may appear. Sudden variation in single characters may also give rise to minor groups, or may change in nature the aspect of the whole lineal group.

In this material I have recognized and determined the extent to which any of the following grades or minor groups are present in any specific form which is to be used for experimental investigation before it is utilized in experimental study. This analysis of the composition of the materials is absolutely necessary and it is only by careful analysis of the composition that is is possible to avoid frequent error and misinterpretation in the progress of investigation. In practice I have found the following scheme of description and analysis to be effective:

PRIMARY GRADE—SPECIES:

A. Gamctic in character, or due to actual factorial composition of the organisms. Changes therein by loss, addition, recombination or transmutation.

1. Species: A group of organisms separable from all other groups by a constant array of qualities, and genetically perpetuated in nature throughout their range of habitats.

SECONDARY GRADE:

a. Habitudinal varieties: A secondary and geographically localized portion of the primary group which is distinguished from the primary group by the possession of difference in, or accentuation of the characters of the primary group in the geographic area inhabited by the differentiated portion of the species, and genetically constant in nature.

b. Recurrent "mutations": Periodic, or recurring discontinuous variations in the group, which appear as sudden deviations, independent, genetically perpetuated races.

c. Saltations (sports): Occasional variation of greater or less magnitude appearing without visible cause and often becoming the basis of races of permanency and of permanent modifications in the specific group. Usually involve sudden change in several associated characteristics. Rare and irregular in occurrence.

TERTIARY GRADE:

d. Biotypes: Lines of descent in which one or more attributes or qualities are accentuated or suppressed and capable of isolation and propagation by breeding. Such biotype groups present intergrading variations with the remainder of the population and the "purification" results in reducing the fluctuation of the type. Equivalent to the Biotype of Johannsen, Elementary Species of DeVries, and many of the "varieties" of domesticated plants and animals.

B. Somatic in character: Not due to factorial change but to developmental alteration of the somatic expression of gametic factors.

a. Habitudinal somatotypes: Somatic alterations recurrently produced in any form in successive generations by influences present in the habitat without producing gametic change. Not inheritable and always lost on change of habitat. Variable. Character only decided by breeding in changed environment.

b. Environmental (aberrations): Irregular discontinuous somatic changes produced by sudden irregularities in the medium or other excessive conditions. Not inheritable. Character of change only proven after breeding tests.

This arrangement of the divisions is applicable to species in a state of nature, where genetic continuity and stability are demanded. Often there are found year after year, associated with some species, certain types, and these are described as varieties or even as species—*e. g., L. melanothorax* Stål—a procedure that is entirely justifiable on the basis of the information ordinarily available; but not infrequently I have found that these types are not genetically continuous, but are produced anew as sports recurring in each generation, or

even as somatic aberrations. It would be interesting to know how many of the rare species varieties and instances of groups of closely allied species described as inhabiting the same area are of this character.

Study of actual material in the field, in life, is absolutely essential for all genetic studies, and this must be done in many localities through a series of years or generations. From different localities samples of the population must be taken, tested by breeding under some standard set of conditions, and these two sets of observations will eventually give the only true basis for the correct determination of the nature and quality of the materials used for experiment. This has been the general and inflexible plan in all of my work.

In its application I have tried to go, and in the main have personally gone, several times over the entire range of each species, collecting data and materials in many localities, and each location was visited again in subsequent years. This has given extensive data of distribution, ecology, and the differences in nature. From many locations materials were tested under standard conditions, and the gametic constitution of the species in the locality determined. This data, plus that from observation in nature, gave the basis for thorough knowledge of the nature of the materials used in this experimental investigation of evolution.

SPECIES AND SUBDIVISIONS OF THE LINEATA GROUP.

In nature I have found and tested the following species and their minor divisions, which I have arranged in two divisions, as follows:

A. Elytral markings edged with an irregular double row of impressed punctations.

Undecimlineata division:

(1) *L. panamensis* n. sp.
L. guatemalensis n. sp.
L. undecimlineata Stål.
Saltation: *abrupta* nov. var.
L. diversa n. sp.
Habitudinal variety: *rugosa* nov. var.
L. angustovittata Jacoby.
L. signaticollis Stål.
Biotype: *nigropunctata* Sturm.

Multitæniata division:

(2) *L. multitæniata* Stål.
(1) Habitudinal variation: *multitæniata* Stål.
Recurrent mutation: *melanothorax* Stål.
Saltation: *tacubayænsis* nov. var.
Biotype: *obscura* nov. var. *multilineata* Stål.
(2) Habitudinal variation: *variabilis* nov. var.
Recurrent mutation: *melanothorax* Stål.
(3) Habitudinal variation: *intermedia* nov. var.
Recurrent mutation: *melanothorax* Stål.
L. oblongata n. sp.
Biotypes: (*a*) red, (*b*) orange, (*c*) yellow.
L. rubicunda n. sp.
L. decemlineata Say.
Saltations: *pallida* nov. var., *defectopunctata, minuta, tortuosa. melanicum, rubrivittata.*

B. Elytral stripes edged with single even regularly placed row of impressed punctations.

Juncta division:

L. juncta Guer.
Habitudinal variation: *texana* Sch.
L. defecta Stål.
L. tumamoca n. sp.

These species are unmistakably distinct, easily separable in life, and while some of them might be further divided to increase the number of species, I can not at present find adequate basis therefor.

In the working over of the literature of the taxonomy only important references and synonyms are retained. Many references are vague, and so many purposeless, incorrect citations and determinations appear that I have omitted all but those necessary to trace clearly the correct name and synonymy of each species.

In the description of these species use must be made of a more precise nomenclature than has hitherto been employed, especially of the elements of the color-pattern which are constant and useful as differentials. In my 1906 paper (p. 60, figs. 1 and 2; p. 143, fig. 7) I give the arrangement and nomenclature of the centers of pattern-formation, which are designated in the main from the sclerite or portion thereof occupied, with the exception of the pronotum, where an empirical lettering is used.

DESCRIPTION OF THE SPECIES.

UNDECIMLINEATA DIVISION.

Elytral markings edged with an irregularly placed double row of impressed punctations.

LEPTINOTARSA UNDECIMLINEATA STÅL.

Myocoryna 11-lineata Stål. Öfv. af. K. Vet. Ak. Förh., 1858, p. 316.2.
Chrysomela undecimlineata Stål. Monog. Chrys. d. Am., 1862, p. 163; Chevolat, Dej. Cat. 3d ed. p. 421; Krantz, Berl. Zeit., 1874, t. I, f. 5.
Leptinotarsa undecimlineata Stål in Gemminger et Herold, 1874, Catalog. Coleop. t. XI, p. 3441; Jacoby, 1883, Biol. Centr. Am., vol. VI, pt. 1, p. 234; Tower, 1906, Inv. Evol. Chrys. Beetles Gen. Lept., pp. 5–14.

I have retained the name *undecimlineata* for the species inhabiting the savannahs and foot-hills around the Gulf of Campeche in Mexico. This is the form originally described by Stål. The habitat Mexico and the flavo-testaceous basal joints of the antennæ possessed by this form indicate clearly that Stål's name should be retained for this species and that *L. diversa, L. guatemalensis* n. sp., and *L. panamensis* should be given equal systematic rank. Stål's description is as follows (Monog. Chrys. d. l.'Am., p. 163):

"Nigra, supra pallide flavescens; basi, apice maculaque media triangulari capitis, maculis liturisque prothoracis nigris; margine inflexo, sutura vittisque quinque elytrorum aeneo-nigris. Long. $8\frac{1}{2}$ — 12, Lat. $5\frac{1}{2}$ — 7 millim.

"Patria: Mexico, Costa Rica, Bogotà, Bolivia. (Mus. Holm., etc.)

"Statura praecedentis. Ovalis, sat convexa, nigra, nitida, supra pallide flavescens vel straminea. Caput parce punctulatum, disco laevisculum, basi, apice maculaque magna media triangulari nigris. Antennae apicem versis sensim nonnihil incrassatae, articulis quinque ultimis subquadratis, clavam vix formantibus. Prothorax elytris nonnihil angustior, antrorsum sensim leviter angustatus, pone medium interdum parallelus, disco parce, subtiliter, utrimque nonnihil densius et distinctius punctatus, litura media ut littera V formata maculisque pluribus lateralibus parvis nigris, angulis anticis mucronatis. Scutellum laeve, æneo-nigrum. Elytra lateribus parallelis, subacervatoseriatim distincte punctata, serie punctorum prima paullo ultra tertiam partem elytrorum extensa, margine inflexo, sutura, vitta suturali anteriore nec non

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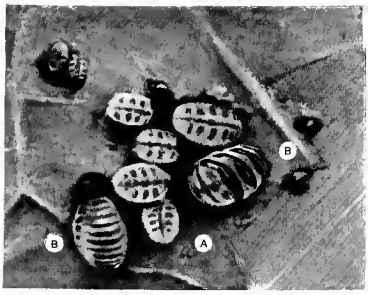

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K. TODA DEL. JULIUS BIEN, N. Y.

COLORED PHOTOGRAPHS OF LIVING MATERIAL OF SOME SPECIES USED IN DIFFERENT EXPERIMENTS.

1. *Leptinotarsa undecimlineata.* Male. × 2.
2. Egg cluster of *L. undecimlineata.* × 1.5.
3. Full-grown larvæ, *L. undecimlineata.* × 1.5.
4. *L. panamensis* n. sp. 4*a*, male; 4*b*, female.
5. *L. guatemalensis* n. sp. Female. × 1.5.
6. Egg cluster of *L. panamensis.* × 4.5.
7, 8. Larvæ of *L. panamensis.* 8*a*, full-grown, 3d stage; 8*b*, 2d stage. × 2.
9. *L. diversa* n. sp. Male and female copulating. × 2.
10. *L. diversa.* Egg cluster and first-stage larvæ. × 1.25.
11. *L. diversa.* Larvæ; (*a*) 2d stage; (*b*) 3d stage, fully grown. × 1.5.

utriusque vittis quinque interstitia alterna occupantibus, tertia et interdum secunda basin attingentibus, intermediis tribus prope apicem abbreviatis, subsuturali et marginali apice conjunctis, nigro-ænesis.

"♂ Segmento ventrali ultimo apice truncato."

DESCRIPTION OF THE LIVING ANIMALS.

Imago (plate 1, fig. 1; plate 2, fig. 8).—Above: Hypodermal color greenish pearly-white in the fully developed imago, with epicranium and pronotum often tinged with pale lemon-yellow; with age color becomes dimmed and browned. Head with sides, mouthparts, and ventral side black; eyes black. Antennæ, basal joint black, second to sixth brown or light yellow brown, with fifth and sixth often with brown color deep or black; seventh to eleventh joints black thickened, longer than broad, terminal joint conical and elongated. Epicranium with anterior lateral epicranial spots united mediad to form heart-shaped spot in center of epicranium, which frequently is prolonged caudad to meet fused posterior lateral epicranial spots in median line, which are always fused mediad. Posterior border and lateral portions behind eyes irregularly punctate with well-marked pits. Pronotum: Constant central **V**-shaped spot composed of $b'+a'+am+pm+a+b$; c' and c always free; often reduced; $d'+e'+f'$ and $d+e+f$ commonly fused, rarely united to central **V**-shaped spot. Posterior border and lateral portion rather thickly and coarsely punctate; anterior and central portion freely punctate. Elytra with 5 longitudinal black stripes with greenish metallic luster, edged with irregular double row of large punctations. Stripes on anal edge unite to form median black stripe where elytra are closed. Epipleuræ inflexed, black, punctate. Below: Legs, mouthparts, thoracic and abdominal surfaces dense shiny black, except that edge of pronotum is of same color as dorsal surface and frequently shows ventral pleural spot, variable.

Size: Not variable; male 7 to 11 mm. long, 5 to 6 mm. broad; female 8 to 14 mm. long, 5 to 7 mm. broad.

Sexes: Female, ventral sclerite of the terminal abdominal segment rounded smooth; male with same sclerite truncate and median groove well defined and reaching well towards middle of plate, variable. In general the male is smaller than the female.

Food: Preserved specimens of the food-plants and live material grown from the seeds were submitted to Dr. Greenman, of the Field Columbian Museum, for identification. Knabe, in 1907, gives the food as *S. torvum,* which, according to Greenman, is confined to the Antilles. It feeds equally well upon *Solanum hertwigi* Bereth (Camaron, Tierra Blanca, San Marcos), *S. diversifolium* Schlecht (San Marcos, Vera Cruz, Coatzocoalcos), and in captivity upon *S. lanceolatum* Cuv., and *S. chrysotrichum* Schl.

JUVENILE STAGES.

Eggs: Laid in bunches upon the lower surface of leaves (plate 1, fig. 2), from 10 to 175 in a bunch; pale yellow in color, with stalk of coagulated gelatinous cement; 1.25 to 2 mm. long, 0.75 to 1.00 mm. broad; flattened and concave on ventral side. Incubation period from 7 to 18 days; average 9 or 10 days.

First larval stages: Head, pronotum, and legs, black; body-spots each with one or more spines. Average length at end of stage 2 to 3.25 mm.

Second larval stage: Head, pronotum, and legs, black; body white or transparent, without spots or spines, except spiracula and wing-spots; white color increasing in intensity with growth, especially in the sternal and pleural portions. Average length at end of stage 5.5 to 6.5 mm.

Third larval stage: In color and markings exactly like second stage, but with body-color ivory white, becoming opaque or yellowed at pupation. Length at maturity, 8 to 14 mm. (plate 1, fig. 3).

Pupa: Pupate in ground, 0.5 to 6 inches below surface. Pupa, pale yellow white, with spiracula spots very small. Duration of pupal stage, 10 to 20 days, average 12 days.

Length of larval period: Range 20 to 42 days, average 27.

Length of ontogeny: On average 49 days, range 30 to 80 days. The larval stages of *L. undecimlineata* Stål described by Dugés are not of this form, but of *L. diversa,* which occurs (rarely) at the location where Dugés obtained his material near Guanajuato.

GEOGRAPHICAL DISTRIBUTION.

In its distribution this species as now limited is confined largely to the savannahs and lower foot-hills and valleys about the Gulf of Campeche (plate 3). I have included here only citation of localities where there is absolute certainty of the correctness of the determination. Many older records, even when the material has been examined, are of no value, owing to poor preservation, and can not be corrected. The records of localities submitted apply only to the form as here limited, and while further information would no doubt change the range and perhaps the relation of these closely placed species, nevertheless the data given here is for a single form, irrespective of its systematic value. I have found it at Campeche (near the city) at Esperanza, Tenabo, state of Campeche; Frontera, Chilapa, San Juan Bautista, Embarcadero, Huimanguillo, state of Tabasco; Coatzacoalcos, Minatilan, Hidalgotilan, Tortugas, Santa Lucretia, Medias Aguas, San Juan Evangelista, in the valley of Rio Coatzacoalcos, and in many other unnamed localities in the same valley; Tlacotalpam, Achotal, San Marcos, Teschoacan, Los Narancos, Chirpo, Perez, El Hule, Tuxtepec, Tierra Blanca, Tepextempa, Motzorongo, Vista Hermosa, Agua Fria, and many other localities in the valley of the Rio Papalaopan and its tributaries; Vera Cruz (near the city), Paso del Macho, Camaron, Misantla—all in the state of Vera Cruz. At all of these localities I have seen this species in all of its stages, and at all of the localities the characters were uniform and constant.

HABITS AND ECOLOGY.

An adequate general account of the habits and ecology of this species is given in my 1906 report (Carnegie Inst. Wash. Pub. 48, pages 14 to 16).

SOURCE OF MATERIAL.

The living material for the various cultures and experiments described herein came in the main from Tierra Blanca, San Marcos, and Coatzacoalcos.

(*a*) The location at Tierra Blanca, Vera Cruz, Mexico, 3 kilometers SW. by W. of the railroad station is on the Coastal Plain at an altitude of 200 feet upon a flat and typical tropical savannah, with an open, park-like, thorn-forest, and poorly developed fringing forest along the water-courses. Well-developed wet (June to January) and dry (January to May) seasons occur here, and the activities of the beetles follow closely the climatic cycle. All material for experimental purposes has been gathered from a restricted locality on the edge of a small stream. Stocks were obtained as follows: No. 700 in August 1903; No. 701 in May 1904; No. 702 in July 1906; No. 703 in June 1908.

(*b*) The location at San Marcos (Vera Cruz, Mexico) is also on the Coastal Plain, at an altitude of 600 feet, in a wide, poorly drained plain, with a short, compact growth of grasses and a well-developed tree-island forest. The climatology is about the same as at Tierra Blanca. All material has been gathered on the edge of a tree island 0.5 kilometer SSW. of the railroad station. Stocks were obtained as follows: No. 710 in May 1904; No. 711 in July 1905; No. 714 in June 1907; No. 715 in June 1908; No. 716 in June 1909.

(*c*) The Coatzacoalcos (Vera Cruz, Mexico) location is 2 kilometers northeast of the town, at the edge of the sand-dunes. It is also a savannah, but modified by the approaching dunes and high humidity brought in from the Gulf of Mexico by the onshore winds. The climatology is modeled on the same plan as at the other two sources of material. Stocks were obtained as follows: No. 720 in May 1904; No. 721 in August 1904; No. 722 in January 1908; No. 723 in August 1908.

Materials from all three localities were, as far as analyses could discover, identical, and not one has thus far shown any gametic characteristics that were strictly limited to any one locality.

SALTATION: L. ABRUPTA NOV. VAR.

***Leptinotarsa angustovitta* Jacoby. Tower, 1906, p. 6, pl. 16, fig. 2, where it is recorded as *angustovittata* Jacoby.**

Imago: Exactly like *undecimlineata* in all respects except that elytral stripes are suppressed or wanting and greenish metallic iridescence gives elytra greenish tinge, or greenish white. In breeding it proves to be a recessive—*i. e.*, absence of stripes is recessive—and breeds true in cultures as an extracted recessive when crossed. It is a rare sport accompanying certain strong climatic influences.

OCCURRENCE.

In nature, Tierra Blanca, 1904, 1907, 1910. Produced in experiment, Chicago, 1905.

JUVENILE STAGES.

Like *L. undecimlineata;* food and habits also identical.

Jacoby's doubt as to the standing and relationship of *L. angustovittata* led me to believe in 1906 that *abrupta* was the same or essentially the same form. I have since 1906 found *L. angustovittata* in nature, reared it, and its affinities are clearly with *diversa* and not with *undecimlineata* Stål.

LEPTINOTARSA DIVERSA N. SP.

***Leptinotarsa undecimlineata* Stål. Material from Orizaba, Oaxaca (Jacoby, 1883); Orizaba, Jalapa, Oaxaca, Mitla, Tlacolula, Tomellin (Tower, 1906), are references to *L. diversa* n. sp. and not to *L. undecimlineata* Stål. Dugés, 1883, Ann. de la Soc. Ent. de Belg., T. 28, pp. 1-6, pl. 1, life-history from Guanajuato, are of forms here designated *diversa*.**

DESCRIPTION.

Imago (plate 1, fig. 9; plate 2, fig. 4).—Above: Head, pronotum, ivory white, more or less tinged with yellowish; pattern of black spots. Elytra, ivory white, often greenish, with 5 dense black longitudinal stripes; costal edge inflexed, black, punctate. Below: Mouthparts and eyes, dense shiny black. Antennæ:

Black, joints 7 to 11, broad as long, distinct, terminal joint reduced, conical, posterior portions of epicranium punctate, with large pits. Pronotal color-pattern uniform, spots and central V-shaped spot constant, composed of $a' + \frac{am}{pm} + a$ and a large spot in each outer posterior angle composed of $d' + e' + f'$ and $d + e + f$. Lateral third of pronotum coarsely punctate, middle third polished, few minute pits, scattered larger punctations.

Size: Larger than *L. undecimlineata* Stål and somewhat smaller than *L. signaticollis* Stål. More robust than former, less so than latter. Male 8 to 14.5 mm. long, 5 to 7.5 mm. broad; female 8 to 17 mm. long, 5 to 9.5 mm. broad.

Sexes: Female, sternal sclerite of last abdominal segment rounded, not truncate; male, same sclerite truncate, with well-marked median groove from posterior border to about the middle of the plate. In general the male is smaller than the female and differs in being narrowed posteriorly, while the female is broadened.

Food: At Orizaba, *Solanum chrysotrichum* Schl.; Dugés (1883) gives *S. torvum;* Dr. Greenman states that *S. torvum* is not found in Mexico; at Guanajuato, on *S. hertwigi* Bereth; on west coast, on *S. diversifolium* Schl. and *S. hertwigi* Bereth.

JUVENILE STAGES.

Eggs (plate 1, fig. 10): Laid on lower surface of leaves in bunches; 20 to 200; yellow, oval, ventral surface slightly flattened and concave; attached by stalk of varying length composed of coagulated gelatinous cement. Long, 1.25 to 2.5 mm., broad, 0.75 to 1.25 mm. Development requires an average of 8 days under normal conditions; range 6 to 12 days. Dugés records eggs of two sorts—first fixed by foot and second stalked. This difference, which exists in nearly all the species of this group, is, as far as I have observed, due entirely to the manner of laying and the drawing out of the material used to cement eggs to leaf into a longer or shorter attaching stalk. It is not a part of the egg.

First larval stage (plate 1, fig. 10): Head, legs, and pronotum, black; body yellow, with full system of spots. Length at hatching averages at end of stage 3 to 4.25 mm.

Second larval stage (plate 1, fig. 11*a*): Head, pronotum, and legs, black; body yellow, due to the fact that body has no hyperdermal pigment; spiracular and wing spots only present. Mid-dorsal line and middle of each segment laterally grayish, owing to lack of fat-body in under-lying structures, giving regular pattern of yellow and grayish on back. Length, 4 to 7.5 mm.

Third larval stage (plate 1, fig. 11*b*): Head, pronotum, and legs, black; terminal part of legs brown; body yellow or chrome yellow, due to increased density of pigment in fat-body. Second to sixth abdominal segments with broad, tergal black band reaching and often fused with spiracular spots. On first abdominal segment, inner and middle tergals only present, rarely united; seventh segment, all spots present, variable. Length at maturity, 9 to 21 mm. Coloration of this stage somewhat variable, due to the fusions of the different tergal centers.

Length of larval life: Varies with condition, but averages 20 days under normal conditions; range, from 12 to 50 days.

Pupa: Pupates in ground from 0.5 to 4 inches in depth, at foot of food-plant. Pupa pale yellow, with small spiracula and tergal markings. Pupal stage lasts on an average of 12 days; range, 8 to 21 days.

Length of ontogeny: Average 60 days in nature; range, 35 to 70 days.

GEOGRAPHICAL DISTRIBUTION.

Mexico (plate 3), on slope of eastern and western escarpment, at altitude of 3000 to 6000 feet; from the state of Vera Cruz, Mexico, at Cerro El Borrego, Cerro Escamela; Sumidero near Orizaba, Jalapa, Cocomatepec, Huatusco; Mitla, Tlacolula, Ejutla, Oaxaca, Villa Alta, state of Oaxaca; Guanajuato (Dugés), canyon of Rio Grande de Santiago, at many points below Cascada de Janacatlán in Jalisco; Tepec, El Cora, territory of Tepec; and it is probably distributed along the Pacific coast to the Gulf of Lower California and south into Oaxaca-Guerrero highlands. On the east it is probably distributed through the foot-hills and north to the Rio Panuco Valley.

HABITAT.

The general distribution of this species is shown in plate 3. It is not a generally distributed form, but, as far as I have observed it, is always local, limited to narrow habitats, either on hillsides, the edges of the steep-walled barrancas that cut the edge of the Mexican Plateau, or in the immediate vicinity thereof. It apparently is distributed along the eastern and western edges of the plateau, on the east as far north as the valley of the Rio Panuco, and on the west it apparently reaches nearly to the head of the Gulf of California; at least, good museum material from the Pacific coast of Mexico is of this type. On the south, in the deserts of the Tehuacan district, it is modified into the geographical variety *rugosa,* and on the Oaxaca-Guerrero highlands it again assumes its typical form. South in Chiapas it occurs typical in form, and apparently it extends southward into Guatemala; at least, museum material from the plateau region of Guatemala is apparently of this same type.

SOURCE OF MATERIAL.

Living material for experiment has come from (a) the east side of Cerro el Borrego and from the southern end of Cerro Escamela near Orizaba, Vera Cruz. Stocks were obtained as follows: No. 815 in August 1903; No. 816 in May 1904; No. 817 in June 1905; No. 818 in August 1907; No. 819 in May 1909. (b) From the canyon of the Rio Grande de Santiago at point due northwest of Guadalajura; in Jalisco known as "la barranca." Stocks were obtained as follows: No. 800 in September 1903; No. 801 in June 1905; No. 802 in May 1907. (c) Two kilometers north from Tlacolula in Oaxaca. Stocks were obtained as follows: No. 810 in June 1904; No. 811 in August 1909.

I have used the material from Cerro el Borrego in the larger part of my experiments. The materials are gametically alike at all three locations.

HABITUDINAL VARIETY RUGOSA NOV. VAR.

(Plate 2, figs. 5, 6, and 7.)

Like *diversa* in form, color, and markings, but larger. Distinguished from *diversa* by coarse punctations of head and pronotum and the very large irregular punctures along the edges of elytral stripes, which are two to five times as large as in *diversa.* Elytral stripes vary from a complete shiny black stripe to almost complete absence thereof. Some examples show only faint brownish tinge in

the stripe, with dense black spots on the edge, and more rarely the stripe breaks longitudinally into two, not unlike *L. angustovittata* Jacoby. All stages occur in the same restricted localities and frequently have been reared in the progeny obtained from a single pair of parents. Sexes distinguished as in *diversa*.

Distribution: Mexico: Desert areas in headwaters of Rio Balsas Valley. Recorded: Teuhuacan, El Riego, Pantzingo, Venta Salada, Mihuatlan (Estado de Puebla), in Guerrero.

Food: S. lanceolatum.

Juvenile stages: Like those of *L. diversa.*

Habitat: A strictly desert form inhabiting some of the most extreme desert areas in America. It is always limited in its habitat to edge of water-courses, either natural or artificial, and its distribution is, therefore, extremely local in character. It has the capacity for surviving through long periods almost free from desiccation by hibernating in earthen cells, which condition, while it apparently allows air to enter, prevents evaporation from the cell, thus retaining the necessary water to enable the animal to pass the critical season.

Source of Materials: All materials in experiment have come from a point 1 kilometer south of El Riego near Teuhuacan in Puebla. Stocks were obtained as follows: No. 900 in May 1907; No. 901 in June 1909.

LEPTINOTARSA PANAMENSIS N. SP.

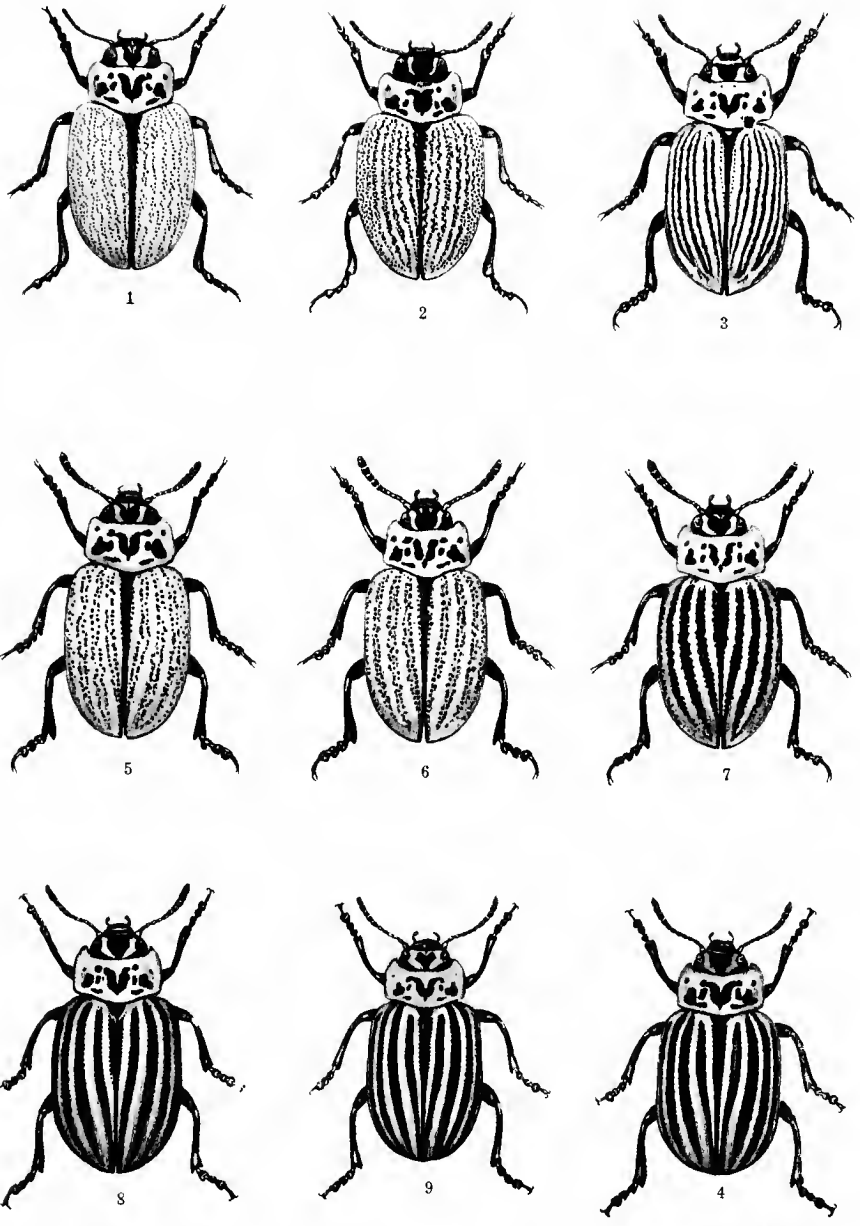
Leptinotarsa undecimlineata Stål, 1862, Monog. Chrys. d. Am., p. 163. Stål gives Costa Rica, Bogotá, and Bolivia as localities for *undecimlineata.* In Panama I have not found *undecimlineata,* but an allied form here described, and in the lower portion of Costa Rica *panamensis* is also the only species present. Dr. Calvert, from Cantago, Costa Rica, sent me all stages of *panamensis.* All references to *undecimlineata* from these regions are therefore probably to *panamensis* as here described. Also quoted by Jacoby, 1882 (Tower, 1906), and many museum specimens in Europe and America from Panama and Costa Rica are so labeled.

Imago (plate 1, fig. 4; plate 2, fig. 9).—Above: Head and pronotum bright yellow, with black markings; mouthparts black with yellowish hairs; elytra ivory white, often with greenish tinge marked with black longitudinal unbroken stripes, 5 or 6 on each elytra, with costal border strongly inflexed, punctate, black. Below: All parts deep, shiny black; hind wings well developed, polished, blackish, deepest on costa and toward base; antennæ, basal four or five joints brownish to yellow, variable, terminal joint short, conical. Seventh to eleventh broad as long and minutely pubescent. Palpi, basal and first joint brownish to yellow, variable; third joint enlarged distally to breadth equal to its length, terminal joint short, conical, black. Head: Anterior lateral epicranial spots fused into heart-shaped spots and prolonged caudad to meet fused portion of lateral epicranial spots. Posterior epicranial border and lateral border to eyes, punctate. Pronotum with all elements present, small, variable, with little or no fusions.

Size: On average smaller than *L. undecimlineata* Stål. Male 6.5 to 11 mm. long, 4 to 6 mm. broad; female 7 to 12 mm. long, 4.5 to 7 mm. broad.

Sexes: Female with sternal sclerite of last abdominal segment rounded, slightly truncate; male with same sclerite sharply truncate, with median groove, variable in length reaching anteriorly. In general the males smaller than the females, and less broadened posteriorly.

Food: Solanum diversifolium.



K. TODA DEL.

JULIUS BIEN, N. Y.

ADULT FORMS OF THE UNDECIMLINEATA DIVISION, IN THEIR LIVING COLORS, BUT IN THE POSES COMMON IN TAXONOMIC LITERATURE.

1. *L. signaticollis.* × 3.
2. *L. signaticollis*, biotype *nigropunctata.* × 3.
3. *L. angustovittata.* × 3.
4. *L. diversa* n. sp. × 3.
5, 6, and 7. *L. diversa* geog. var. *rugosa* nov. var. × 3.
8. *L. undecimlincata.* × 3.5.
9. *L. panamensis* n. sp. × 3.

JUVENILE STAGES.

Eggs (plate 1, fig. 6): Laid on lower surface of leaves in bunches, from 10 to 250; pale, yellowish white, oval, ventral surface slightly concave, attached by stalk of gelatinous cement. 1.5 to 2.25 mm. long, 0.60 to 1.20 mm. broad. No sculpture. Development requires from 7 to 15 days, depending upon temperature and rate of evaporation.

First larval stage: Head, legs, and pronotum shiny black; body pale transparent yellow, with fully developed system of color markings, each spot provided with one or more spines. Length at end of stage 2 to 3.5 mm. Closely resembles the same stage in *L. undecimlineata* Stål, *L. signaticollis* Stål, and *L. diversa* n. sp.

Second larval stage (plate 1, fig. 8*b*): Head, legs, and pronotum shiny black; body, well-developed row of spiracula spots and with tergal plates of last two abdominal segments black below and pleuræ yellow, due to yellow color of fat-body below the transparent integument; tergal portions of thorax and abdomen grayish, due to transparent integument and absence of fat-body in median line. Length at end of stage 4 to 6.5 mm.

Third larval stage (plate 1, figs. 7 and 8*a*): Markings and color precisely same as in second stage, yellow color (due to fat-body) increasing throughout this stage until at the end of the tergal gray area is entirely or nearly replaced by yellow, often becoming a deep lemon yellow. Length at end of stage 10 to 18 mm.

Length of larval stage: Varies with the conditions of growth, food, temperature, etc.; averages 25 days, range from 15 to 35.

Pupa: Pupates in ground at foot of plant, 1 to 6 inches from surface. Pupa pale yellow with yellow white spiracula spots. Pupal stage lasts from 8 to 14 days; average 10 days.

Length of ontogeny: Average 56 days; range 36 to 64 days.

GEOGRAPHICAL DISTRIBUTION.

Panama, Costa Rica, south to Isthmus of Darien. I have collected and tested material of this species and find it to be genetically constant from Corozal, Pedro Miguel, Tabernilla, Gatun, Colon, Balboa, Cerro Ancon, in the Canal Zone, Panama; Bocas del Toro, Changuinola Junction, and numerous points in Sexola Valley in Costa Rica. I also have dead material that was not tested by breeding from the lower portion of Panama, valley of the Rio Succio, from Cartago in Costa Rica (coll. by Dr. P. P. Calvert), and other dead material from Costa Rica which is identical. I have, therefore, on plate 3 given in solid color the distribution of the tested materials and the probable distribution in dotted color, as far as I can, of this species over lower Central America.

HABITAT AND ECOLOGY.

Little comment concerning the ecology of this form is needed, and while more general in habitat distribution than the two preceding species, it is, nevertheless, limited in its distribution and ecology by the same factors and in the same manner as is *L. undecimlineata* Stål. Its more widespread habitudinal occurrence within its range is due entirely to the topographical and climatic character of the area of distribution, rather than to differences of habit or of ecological complex demanded.

SOURCE OF MATERIAL.

Materials utilized for experimentation were obtained at Pedro Miguel (stock No. 1705, obtained August 1912), about 1 kilometer north of village; at Corozal (stock No. 1700, obtained August 1912), near railroad station; at Gatun (stock No. 1710, obtained August 1912; No. 1711, obtained September 1913), and 2 kilometers south of the railroad station, and at Culebra (stock No. 1715, obtained August 1912; No. 1716, obtained September 1913). Habitats fairly uniform for all, but at the Gatun location, in the hills, it rains almost the entire year, while at Corozal a fairly well-marked dry season occurs between January and May.

LEPTINOTARSA ANGUSTOVITTATA JACOBY.

Leptinotarsa undecimlineata Stål var. Jacoby, 1883, Biol. Centr. Am., vol. VI, pt. 1, p. 234. Described as a variety.
Leptinotarsa angustovittata Jacoby, 1891. Biol. Centr. Am., vol. VI, pt. 1, suppl., p. 254, pl. XLI, fig. 15.

This species, in its distribution, habitat, as well as its structural and color characters, and in its juvenile stages, is clearly more closely related to *L. diversa* than any other species in the group. Its occurrence in semidesert habitats, and the fact that it is often closely approximated by the geographical variety of *L. diversa* (*rugosa* nov. var.) lead me to suspect that it is either a desert habitudinal variety of *L. diversa* or a modification thereof that has become permanent in semidesert locations. The data is not available to reach a decision of the relationship, and I have therefore allowed it to stand provisionally as an independent species. It is possible also that this form may have arisen and is now arising through hybridization, at any rate I have produced in experiment by hybrid reactions a form that I am not able to differentiate from Jacoby's *L. augustovittata*. Stock from nature at Guanojuato breeds genetically true, even under changed environment. Jacoby's description (Biol. Centr. Am., vol. VI, pt. 1) is as follows:

"*Leptinotarsa undecimlineata*, Huj. *op.* (*partim*).

"Black; the head with two flavous spots; the thorax and elytra flavous, the former spotted with black, the latter with eight narrow black, strongly punctured, longitudinal stripes. Length, 5–6 lines. Habitat: Mexico, Guanajuato (Sallé), Morelia, and Tacambaro in Michoacan (Höge), Xucumanatlan in Guerrero (H. H. Smith).

"This insect was previously treated by me as a variety of *L. undecimlineata*, but as we now have received twenty additional specimens, all alike, I am compelled to treat it as a distinct species. It agrees in everything with *L. undecimlineata*, except that the elytra have eight very narrow black stripes, each of which is abbreviated at a short distance before the apex; these stripes are deeply punctured in single, sometimes in double and very closely contiguous rows, this character separating the species from *L. undecimlineata*, in which each elytron has four much broader stripes, and the stripes singly or doubly punctured along their margins."

DESCRIPTION OF LIVING ANIMALS.

Imago (plate 2, fig. 3).—Head: Pronotum, greenish ivory white, often yellowish, marked with black spots; eyes and mouthparts shiny black; elytra grayish white or grayish-gray white, with 4 pairs of closely placed parallel black lines, strongly punctured. These are equivalent to the elytral stripes of

other species, except that in this species the central portion of each stripe is wanting and only the punctate margins thereof are pigmented. Even in this there is considerable variability; examples are not uncommon in which the pigment is almost entirely lacking, giving an appearance much like *L. signaticollis* Stål var. *nigropunctata* Sturm. Color-patterns of head and pronotum precisely like *L. diversa* in pattern, fusions, and variations. Antennæ black; last five joints as broad as long, terminal joint conical. Below: Shiny black; under wings, pale blackish brown, densest at base and along the costal edge. The full extent of coloration is not attained until 3 to 5 days after emergence.

Size: Male 7 to 14 mm. long, 5 to 7.5 mm. broad; female 7 to 18 mm. long, 5 to 8.25 mm. broad.

Sexes: Female with central sclerite of last abdominal segment rounded smooth; male with the same sclerite truncate and grooved to about the middle of the plate.

Food: Solanum hertwigi Bereth., *S. diversifolium* Schl., and *S. lanceolatum* Cuv.

JUVENILE STAGES.

In all live material that I have seen these stages are indistinguishable from the corresponding stages of *L. diversa.* Length of larval periods and habits identical. Pupates deeper than *L. diversa—i. e.,* 3 to 8 inches.

GEOGRAPHICAL DISTRIBUTION.

(Plate 3.)

Mexico, Guanajuato (Sallé), State of Guanajuato; Morelio and Tacambaro, State of Michoacan (Höge); Xucumanatlan, State of Guerrero (Smith), Jacoby, 1891. I have found it in the State of Guanajuato at Guanajuato, Irapuato, Dolores Hidalgo, and at Morelia, Zitacuaro, Uruapan, Tinguindin, State of Michoacan.

HABITAT.

Ecologically this species presents little of interest. Its habitat for breeding and hibernation is almost identical in kind and conditions with that of *L. diversa,* excepting that it is uniformly more arid, less water present, with a longer dry season, and higher soil temperature at the end of the dry seasons.

SOURCE OF MATERIAL.

This species has not been used to any extent in experiments thus far, but material has been reared and tested in the laboratory from Dolores Hidalgo in Guanajuato (stock No. 1416 obtained in June 1908), Morelia (stock No. 1400 obtained in August 1905; No. 1401 in July 1907), and Uruapan in Michoacan (stock No. 1416 obtained in June 1907; No. 1417 in June 1908), Mexico.

LEPTINOTARSA SIGNATICOLLIS STÅL.

Myocoryna signaticollis Stål. Of. Vet. Ak. Förh., 1859, p. 317.5.
Chrysomela signaticollis Stål. Monog. Chrys. Am., 1862, p. 163.
Leptinotarsa signaticollis Stål. Gemminger et Harold, 1874, Catalog. Coleop., t. XI, pt. 1, p. 3441; Jacoby, Biol. Centr. Am., Jan., 1883, vol. VI, pt. 1, p. 232, pl. XIII, fig. 20, suppl., May 1891, p. 253; Tower, Inv. Evol. Chrys. Beetles Gen. *Leptinotarsa,* 1906, p. 6. Var. *nigropunctata* Sturm, in literature; Gemminger et Harold, 1874, Catalog. Coleop. t. XI, pt. 1, p. 3441, given as syn.; Jacoby, Biol. Centr. Am., 1883, vol. VI, pt. 1.

There is no difficulty in the recognition of this species, even in badly preserved museum material, but the variety *nigropunctata* Sturm, is a biotype, breeding true in its distinctive character, and behaves as an alternative dominant character in heredity. Stål's description of *L. signaticollis* (Monog. Chrys. Am., p. 163), is as follows:

"Nigra, supra sordide flavescens; maculis prothoracis capiteque nigris, hujus vittis duabus flavis; elytris subvage nigro-punctatis, vitta angusta suturali margineque inflexo æneo-nigris. Long. 12, Lat. 7 millim.

"Patria: Mexico. (Mus. Holm., Coll. Dohrn). Ovalis, sat convexa, nigra, nitida. Caput parce punctulatum, vittis duabus obliquis maculisque duabus minutis ante medium sordide flavis. Antennæ articulis quinque ultimus leviter transversis, clavam minus distinctam formantibus. Prothorax sordide flavescens, elytris nonnihil, angustior, lateribus parallelis, antice angustatus, disco parce, subtiliter, utrimque paullo densius et distincte punctatus, litura media ut littera V formata maculisque lateralibus pluribus, plus minus confluentibus, nigris. Scutellum læve, nigrum. Elytra lateribus parallelis, dilute sordide flavescentia, vage distincte nigro-punctulata, punctis hic illic in series dispositis, vitta angusta suturali margineque inflexo æneo-nigris."

This description applies very well to the dead dried specimens of collections. The following description is from living material in all stages:

Imago (plate 2, fig. 1).—Above: Hypodermal color of head and pronotum pale grayish white, often pale yellowish ivory-white, becoming darkened with age; elytra, pale gray or pale gray-green. Head, mouthparts black; antennæ black, terminal joint truncate, eighth to eleventh thickened, broader than long. Epicranium with anterior lateral epicranial spots fused medianward to form heart-shaped spot, and prolonged caudad to fuse with posterior lateral epicranial spots, which are united mediad. Posterior border and lateral portions of epicranium behind eyes irregularly punctate with fairly well marked pits. Eyes black. Pronotum with variable pattern of black spots. $b'+a'+\overset{am}{\underset{pm}{+}}+a+b$ are always fused to form widely open **V**-shaped median spot. *c'* and *c* always free, and *d, e, f* groups somewhat variable. Margins, especially anterior lateral portions, punctate, with well-defined pits; central portion with few minute pits irregularly distributed. Elytra with black anal edge giving median black stripe when closed. Each elytron with ten irregularly placed double rows of punctations, marking position of chitinous columns between elytral lamellæ, and with slightly variable amount of black pigment in bottom of each pit. These rows are arranged in a definite pattern shown in plate 2, figs. 1 and 2. Under wings, pale yellowish brown, transparent, darker towards the base. The adult coloration is not attained until about the third day after emergence.

Size: Male 7 to 13 mm. long, 6 to 7.5 mm. broad; female 7.5 to 14.5 mm. long, 7 to 9 mm. broad.

Sexes: Female with sternal sclerite of last abdominal segment rounded; male, same sclerite sharply punctate, with median groove reaching nearly to anterior border.

Food: At Cuernavaca, *Solanum hertwigi* Bereth., *S. diversifolium* Schlecht. At Atlixco and Matamoros, *S. diversifolium* and *S. lanceolatum* Cuv. Will also feed upon *S. chrysotrichum* Schl., but will not eat *S. torvum* from the Antilles. Identification of food-plants by Dr. Greenman.

JUVENILE STAGES.

Eggs: Yellow, rarely lemon-yellow or yellowish white, laid on lower surface of leaves, in bunches of 5 to 200. Length, 1.75 to 2.5 mm.; breadth, 0.75 to 1.35 mm. Concave on ventral side and attached to leaf by a stalk of greater or less length composed of a coagulated gelatinous material which is extruded ahead of eggs at laying. Development requires from 5 to 12 days, depending upon external conditions.

First larval stage: Head, pronotum, and legs black; body yellow, with color-pattern shown in plate 7, figure 10*a*. Tergal spots all with one or more spines. Length at hatching, 2.25 mm.

Second larval stage: Head, pronotum, and legs black; body yellow, with single row of black spiracula spots (plate 7, fig. 10 *b*). Length, 4 to 7.5 mm.

Third larval stage: Head, pronotum, and legs black; body yellow, with two rows of spots on pleurum, spiracula, and baso-pleural; inner, middle, and outer tergal spots present; more or less fused lateralward; frequently fused with spiracula spots; inner tergal always fused antero-posteriorly on each segment (plate 7, fig. 10*c*). Length, 10 to 19 mm. Length of larval period varies with meterological conditions, food, and the local race; may be passed over in 15 days or prolonged to 40 or 50. In nature and in cultures it averages about 25 days.

Pupa: Pupates in ground at foot of plant, from 0.5 to 5 inches deep, depending upon soil hardness, moisture, and air-supply. Pupal stage lasts from 10 to 30 days; average 15 days. Of this period 25 per cent is passed in prepupal stage, 60 per cent as pupa, and 15 per cent as imago. Length of ontogeny from egg laying to adult averages 40 days; range 28 to 70 days.

GEOGRAPHICAL DISTRIBUTION.

(Plate 3.)

Mexico, Stål, 1859, 1862; Gemminger et Harold, 1874; Jacoby, 1883; Tower, 1906. Recorded from Izuca, Puebla, State of Puebla (Sallé; Jacoby, 1883); Cuernavaca, State of Morelos; Aurula, Xucamanatlan, State of Guerrero (H. H. Smith; Jacoby, 1883); Alarcon, Cuanutla, Jojutla, State of Morelos; Matamoros de Izucas, Atlixco, Tatetla, State of Puebla (Tower, 1896).

The habitat of this species is confined to the upper portion of the eastern and northern branches of the Rio Balsas, and in the main lives close to the foot of the great Mexican escarpment (plate 23). I have not found it to extend northwestward beyond the Rio Balsas Valley, eastward beyond the Rio Coetzala Valley; neither does it reach an altitude of over 6000 feet, rarely above 5500 or below 4000. It is narrowly limited in range, and is also still more restricted in its ecological relations.

SOURCE OF MATERIAL.

Of this species my chief source has been from localities near Cuernavaca, Morelos, Mexico: (*a*) Rancho Basoco colony: Stock No. 418, obtained July 1903; No. 419, May 1904. (*b*) Quauhtemotzin colony: Stock No. 417, obtained July 1903. (*c*) San Antonio colony: Stock No. 420, obtained May 1904; No. 421, July 1905; No. 422, June 1906. Other material has been used at different times from near Atlixco and Matamoros in Puebla. The chief stock location has been that at Rancho Basoco.

BIOTYPE NIGROPUNCTATA STURM.

Similar to *signaticollis* (plate 2, fig. 2), but with elytra having numerous large black punctations, which may or may not show arrangement in pattern of ten irregular double rows; punctures often confluent, giving the appearance of longitudinal vittæ. Appears also in experiment, most often as a dominant, and when isolated breeds true; female sex-linked, but not uniformly so. (See also Jacoby, 1883, p. 232, who had Sturm's specimens for study.) Recorded from Cuernavaca, Cuautla, Estado de Morelos, Atlixco, Estado de Puebla.

MULTITÆNIATA DIVISION.

LEPTINOTARSA MULTITÆNIATA STÅL.

Myocoryna multitæniata Stål, 1859. Ofv. af K. Vet. Ak. Förh., p. 317.4.
Chrysomela multitæniata Stål, 1862. Monog. Chrys. Am., p. 164.
Leptinotarsa multitæniata Stål. Gemminger et Harold, 1874, Catalog. Coleop., t. IX, pt. 1, p. 3441; Jacoby, 1883, Biol. Centr. Am., vol. VI, pt. 1, p. 253; Tower, 1906, Inves. Evol. Chrys. Beetles, Gen. Lept., Carnegie Inst. Wash. Pub. No. 48, pp. 7, 18.

This species has given me more trouble in the effort to untangle its taxonomic relations than all of the others combined. It is a plastic, variable, polymorphic form, showing curious phenomena of heterogeneity which I shall discuss later. From present data I have arranged the species and its minor forms as follows:

L. multitæniata Stål.
- Habitudinal variation: *multitæniata* Stål.
  - Recurrent mutation: *melanothorax* Stål.
  - Saltation: *tacubayaensis* nov. var.
  - Biotypes:
    - *multilineata* Stål.
    - *obscura* nov. var.
- Habitudinal variation: *intermedia* nov. var.
  - Saltation. *melanothorax* Stål.
- Habitudinal variation: *variabilis* nov. var.
  - Recurrent mutation: *melanothorax* Stål.
  - Biotypes:
    - Red hypodermal color.
    - Orange hypodermal color.
    - Yellow hypodermal color.
    - White hypodermal color.

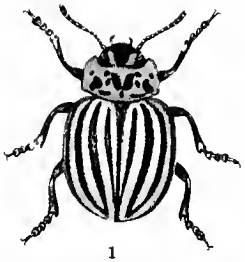
HABITUDINAL VARIETY L. MULTITÆNIATA-MULTITÆNIATA STÅL.

Stål's description of the typical *multitæniata* form, taken from his monograph (1862), is as follows:

"Ovalis, supra flavescens, subtus cum antennis, pedibus, maculis prothoracis, limbo scutelli, sutura vittisque quinque, exteriore intramarginali, elytrorum, nigra; maculis pectoris limboque apicali segmentorum ventris flavis; elytris minus regulariter seriatim punctatis. Long. 10, Lat. 6⅔ millim.

"Patria: Mexico (Mus. Berol., Coll. Dohrn).

"*C. multilineata* simillima, prothorace latiore, elytris regularius seriatim punctatis differt. Ovalis, sat convexa, nigra, nitida. Caput sat dense, distincte punctatum, vittis duabus obliquis, basi conjunctis, flavis. Antennæ articulis quinque apicalibus transversis, clavam sat distinctam formantibus. Prothorax elytris nonnihil angustior, sat convexus, antrorsum sensim, angustatus, disco parce, subtiliter, utrimque densius, subfortiter punctatus, flavescens, maculis compluribus lateralibus lituraque media ut littera V formata, plus minus confluentibus, nigris ornatus. Scutellum flavescens, plus minus late nigro-limba-



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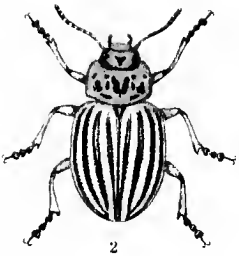

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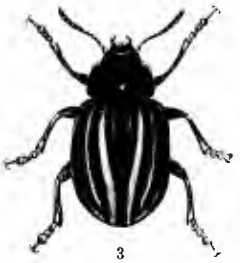

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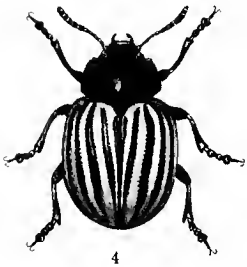

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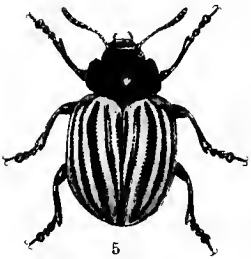

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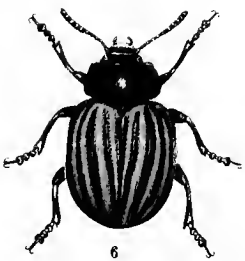

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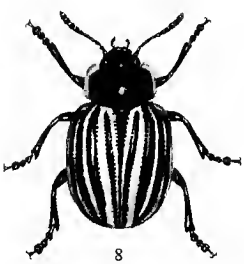

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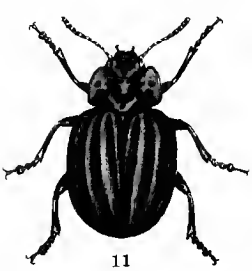

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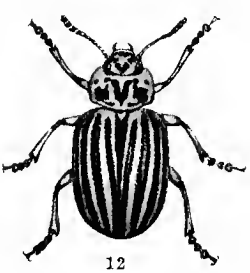


12

K. TODA DEL.

JULIUS BIEN, N. Y.

1. *L. multitæniata.* Typical form. × 2.
2. *L. multitæniata,* biotype *multilineata.* × 2.
3. *L. multitæniata,* saltation *tacubayænsis* nov. var. × 2.
4, 5, and 6. *L. multitæniata* recurrent mutant *melanothorax,* shown in three biotypical forms, yellow, orange, and red, in which it may occur. × 2.
7. *L. rubicunda* nov. var. × 2.
8. *L. multitæniata,* biotype *obsoleta* nov. var. × 2.
9. *L. multitæniata* geog. var. *intermedia.* × 2.
10, 11. *L. multitæniata* geog. var. *variabilis* nov. var. × 2.

tum, læve. Elytra flavescentia, lateribus parallelis, minus regulariter seriatim vel subacervato-seriatim distincte punctata, linea suturali basin versus latiore nec non vittis interstitia alterna occupantibus, nigris, vitta interstitii decimi medio et ante medium a margine paullo remota, interstitio nono reliquis fere duplo latiore, medio vage, remote et distincte punctato; margine inflexo posterius nigro. Pectus maculis nonnullis plus minus distinctis flavo-testaceis. Segmenta ventris apice plus minus late flavo-limbata."

HABITUDINAL VARIETY MULTITÆNIATA-MULTITÆNIATA STÅL.

(Plate 4, fig. 1.)

I have reserved this name for that portion of the entire species which is limited to the southern end of the Mexican Plateau and which agrees with Stål's descriptions, and is also the form described and recognized by Jacoby, 1883-1891, in the Biologisches Centrallblatt under this name.

Imago: Oval, convex, robust in form. Above: Head and pronotum light tan, often with tinge of red marked with black color-pattern. Epicranium and pronotum punctate, especially on lateral and posterior portions, with irregularly arranged variable punctures. Elytra pale yellow white or yellowish, rarely reddish yellow, marked with five longitudinal black stripes, which are edged by an irregular double row of impressed punctations, variable in size. Costal edge inflexed, dark brown or black, slightly grooved and finely punctate, sparingly or smooth. Second and third elytral stripes often united posteriorly, and may also be united to fourth stripe. Eyes black, epicranium with anterior lateral epicranial spots fused and reaching to anterior border and to fused posterior lateral epicranial spots posteriorly. On pronotum $a'+b'+\begin{matrix}am\\+\\pm\end{matrix}+a+b$ usually fused to form broadly open V-shaped spot, *c* usually free, and *d' e' f'* and *d e f* group variable, usually united, and often forming extensive fusions with other parts of pattern. Below: Uniform light tan-color or brownish yellow marked with black, variable from all color-centers distinct to completely black. Legs: Femora dark brown or black, tibia, ends always black, central portions brown or black, sometimes reddish brown, variable. Entire ventral surface and legs punctate, irregular, variable, slightly pubescent.

Size: Variable, depending upon food and climatic conditions. Females always larger than males. Female, 7 to 13 mm. long, 6 to 9 mm. broad; male, 6 to 12 mm. long, 5 to 8 mm. broad.

Sexes: Female with ventral sclerite of last abdominal segment rounded flat; male with same sclerite truncate, slightly grooved; female also more robust and broadened posteriorly than male.

Food: Feeds exclusively in nature upon *Solanum rostratum* Dun., or close Mexican allies thereof, on *S. elæagnifolium,* and more rarely upon *S. tuberosum,* and very rarely upon other Solanaceæ. *S. rostratum* is its food. In captivity, when deprived of other food, will eat *S. diversifolium, lanceolatum,* and *hertwigi.*

JUVENILE STAGES.

(Tower, 1906, plate 17, figs. 10, 11, 12.)

Eggs: Bright yellow, sometimes pale yellow, laid upon the lower surface of the leaves, in bunches of from 5 or 6 to 200, and cemented to the leaf by a coagulated gelatinous cement; oval, flattened and concave ventrally, smooth, polished. Length, 2.5 to 3.25 mm.; breadth, 1.3 to 1.9 mm.; incubation, 5 to 10 days, depending upon temperature and moisture conditions.

First larval stage: Head, pronotum, and legs, black; body yellow, with full system of body color-markings, each with one or more spines. Length at end of stage, 3.5 to 4.5 mm.

Second larval stage: Head and legs black, pronotum with posterior edge black, anterior border and sides to middle, yellow or brown; body bright yellow, ornamented with spiracula, wing and basi-pleural spots, and a variable number of the anterior members of the inner tergal spots. Length at end of the stage, 6.5 to 8.25 mm.

Third larval stage: In color and pattern identical with the second. Length at end of the stage, 10 to 18 mm. Length of larval period varies from 13 to 25 days, average 17 days; but much depends upon surrounding conditions of growth.

Pupa: Pupates in ground, from 2 to 6 inches in depth; pupa yellow. Period of pupation, 6 to 15 days, but average about 9 days.

Length of ontogeny: From 19 to 40 days; usual average is between 25 and 30 days. The larval stages are very constant in this geographical variety and all of its minor divisions.

HABITAT AND ECOLOGY.

This form passes the winter—*i. e.,* the dry season, from the middle of September to May—in hibernation in the ground as an imago. It emerges from hibernation in May, usually about the 15th, in the region of Mexico City, and begins breeding about June 1 to 15, depending upon the advent of the rainy season. It produces a brood that becomes adult about July 20, which in turn breed at once and give a second brood, which emerges about the end of August. These latter feed for a few days, and with the cessation of the rains and cooler nights of September, enter into hibernation, and by September 15 or October 1 they have all gone into hibernation, with the exception of a few that never do enter the ground. These latter are curious hybrid forms produced in the following manner: Some members of the overwintering hibernating population may remain alive and breed with their descendants, giving a cross of *summer form* × *winter form,* thus producing hybrids which are variable in their breeding activities and some of which are extracted summer forms in F_2 and do not hibernate, and are, therefore, eliminated by starvation and desiccation during the dry season.

GEOGRAPHICAL DISTRIBUTION.

The range of this habitudinal variety and its minor divisions is, as indicated on plate 5, limited to the southern end of the Mexican Plateau, between the altitudes of 5000 and 8000 feet. It is especially characteristic of and common in the valley of Mexico (7400 to 8000 feet), the plains of Apam, and eastward to the edge of the plateau (6000 to 8000 feet), the plateau portion of the State of Puebla, especially the headwaters of Rio Atoyac, and less common on the high plateau of the portion of the valley of the Rio Lerma and westward to edge of plateau; northward it passes over into northern habitudinal variety *intermedia,* in the region of the headwaters of the Rio Panuco.

Stål gives Mexico as its habitat; Jacoby, 1883, 1891, gives Toluca, La Parada (Sallé), Cerro de Plumas (Höge); Tower, 1916, *l. c.,* records it from Puebla, Atlixo, Matamoros de Izecas, San Marcos, in the State of Puebla; Apizaco and

Tlascalo in the State of Tlascala; Mexico City, Guadalupe, Tacubaya, Tacuba, Santa Fe, San Angel, Tlalpam, Tlalnepantla, Texcoco, and Mixcoco in the Valley of Mexico.

SOURCE OF MATERIAL.

The stocks for different experimental purposes have been derived as follows, and in the body of the paper will be referred to according to the name of the location from which they were derived.

(*a*) *Guadalupe stock:* From a location on the shore of Lake Texcoco, on an old shore-line east of the Shrine of Guadalupe. Location described, Tower (1906), plates 5 and 6, pages 18 to 20. Improvement and cultivation destroyed this location in 1907-8. Stocks were obtained as follows: No. 500 in July 1903; No. 501 in May 1904; No. 502 in August 1904; No. 503 in July 1905; No. 504 in August 1908.

(*b*) *Chapultepec stock:* From the plain to north of the Cerro Chapultepec, in waste places and pastures; is fast being built up and the location is now nearly obliterated. Stocks were obtained as follows: No. 540 in August 1903; No. 541 in May 1904; No. 542 in July 1905; No. 543 in August 1906; No. 544 in June 1909; No. 545 in August 1908.

(*c*) *Tlalnepantla stock:* From west shore of Lake Xico, near Tlalnepantla. Stocks were obtained as follows: No. 510 in August 1903; No. 511 in May 1904; No. 512 in June 1907.

(*d*) *Puebla stock:* From open plain to northeast of the city, about one-half mile north of Cerro de Guadalupe. An open rolling plain, more or less cultivated; well drained. Stocks were obtained as follows: No. 515 in August 1903; No. 516 in May 1904; No. 517 in June 1905; No. 518 in August 1906; No. 519 in June 1909; No. 520 in June 1910.

(*e*) *Apam stock:* From the open plain of Apam, on the edge of a wet-season lake about a mile northeast of the town. Stocks were obtained as follows: No. 525 in August 1904; No. 526 in August 1905; No. 527 in June 1909.

(*f*) *Chalcicomula stock:* On the road from Chalcicomula to San Andres Chalcicomula, a mile from the plaza; location is a cold, dry upland savannah. Stocks were obtained as follows: No. 530 in July 1904; No. 531 in June 1905; No. 532 in August 1906; No. 533 in June 1908; No. 534 in June 1910.

These stocks and several others have been tested, analyzed, and made the basis of experiments in many lines, and the data of these analyses have entered largely into the understanding of the real nature of this species as it is found in nature, and without the analysis of the laboratory a correct understanding would be impossible.

RECURRENT MUTATION L. MULTITÆNIATA-MELANOTHORAX STÅL.

This form has hitherto been recorded as a species and is so included in all lists and collections. In 1906 I was noncommittal as to its status, but further study shows in the clearest possible manner that it is not a species, but a recurrent mutant, as here recorded.

Myocoryna mulanothorax Stål, 1859. Ofv. af. k. Vet. Förh., p. 317.7.
Chrysomela melanothorax Stål, 1862. Monog. Chrys. Am., p. 166.
Leptinotarsa melanothorax Stål in Gemminger et Harold, 1874, Catalog. Coleop., t. IX, pt. 1, p. 3441; Jacoby, 1883, Biol. Centr. Am., vol. VI, pt. 1, p. 234, suppl., p. 254; Tower, 1906, Inves. Evol. Chrys. Beetles, Genus Lept., Carnegie Inst. Wash., Pub. No. 48, p. 720.

Stål's description is as follows:

"Ovalis, nigra; prothorace læviusculo; elytris testaceo-flavis, minus regulariter geminato-seriatim distincte punctatis, margine inflexo, vitta angusta suturali interstitiisque alternis nigris. Long. 9, Lat. 6½ millim.

"Patria: Mexico (Mus. Holm., Coll. Dohrn).

"Ovalis, sat convexa, nigra, nitida. Caput parce, subtiliter punctulatum. Antennae graciles, articulis quinque ultimis subtransversis, clavam sat distinctam formantibus. Prothorax elytris nonnihil angustior, antrorsum sensim leviter angustatus, lævis, utrimque punctulis raris adspersus, angulis anticis subacutis. Scutellum laeve. Elytra lateribus parallelis, minus regulariter geminato-seriatim distincte punctulata, testaceo-flava, margine inflexo, vitta angustissima suturali nec non vittis quinque, interstitia alterna occupantibus, nigris, vittis basin haud attingentibus, tribus interioribus prope apicem abbreviatis et conjunctis.

"♂ Segmento ventrali ultimo apice truncato."

Easily distinguished by black head and pronotum and black ventral surface. Jacoby, 1883, noted that third and fourth elytral black stripes are sometimes united. This is not common and is due to crossings with another form, *tacubayaensis* nov. var., and its presence in the strain. The elytral ground-color is variable, dark ocher-yellow, yellow, or red. These differences can be separated in breeding, giving the *melanothorax* type in four minor biotypes: *L. multitæniata-melanothorax* b_1, red elytral ground (plate 4, fig. 6); *L. multitæniata-melanothorax* b_2, orange elytral ground (plate 4, fig. 5); *L. multitæniata-melanothorax* b_3, yellow elytral ground (plate 4, fig. 4); *L. multitæniata-melanothorax* b_4, white elytral ground. All of these breed true genetically, and are alternative in their behavior in crossing. (Plate 4, figs. 4, 5, and 6.)

Food, juvenile stages, distribution, habits, and ecology as in *L. multitæniata* Stål.

SALTATION L. MULTITÆNIATA-TACUBAYAENSIS NOV. VAR.

(Plate 4, fig. 3.)

Like *L. melanothorax* Stål in size, form, head, and pronotum; elytra orange or red, with longitudinal stripes, broad brownish black, the first confluent with anal edge, forming large wedge-shaped median stripe, the second broad, united to fused third and fourth stripes posteriorly. Costal edge black, inflexed portion yellow brown and sharply grooved. Impressed punctations, large, 2 to 3 rows, interspaces smooth, arched, conspicuous. Below, all parts shiny black, except posterior and lateral portions of sternal sclerite of abdominal segments. Antennæ second and third joints brown or yellowish, remainder black.

This rare sport breeds genetically true and is in cultures different from *L. melanothorax*. In nature examples of this form are frequently contaminated by attributes from other portions of the species, but no difficulty is encountered in purifying it and establishing it as a stable, uniform race.

Food, juvenile stages, habits, and ecology: The same as in general species.

Geographical distribution: Valley of Mexico; I have found it at Tacubaya, Tacuba, Chapultepec, and Texcoco.

Further complications are introduced by the presence of a biotype form that occurs in the species and with regularity in some locations, to which I have given Stål's name, *L. multilineata,* in that Stål's description fits perfectly the biotype in question. In museum material it is frequently not unlike some specimens of *L. decemlineata* Say and *L. oblongata,* but it has no relations to these two

species, and it further occurs in the region from which Stål's material probably came. I have considered it best to retain his name for this division of the species.

BIOTYPE L. MULTITÆNIATA-MULTILINEATA STÅL.

(Plate 4, fig. 2.)

Myocoryna multitæniata Stål, 1859. Ofv. af. K. Vet. Ak. Förh., p. 316.3.
Chrysomela multitæniata Stål, 1862. Monog. Chrys. Am., p. 165; considered as synonym of *L. decemlineata* Say, and apparently confused with it; Harold, 1874, Ber. Ent. Zeit., p. 444, synonym with *decemlineata;* Jacoby, 1883, Biol. Centr. Am., also synonym with *decemlineata*, following Stål, gives Mexico and Costa Rica as habitat of *decemlineata* incorrectly.

Stål's description, which follows, taken from his monograph (Chrys. d. l'Am.), fits perfectly the portion of *multitæniata* designated here as a biotype:

"Ovalis, pallide flava, subtus cum capite prothoraceque nigro-maculata, hoc sat convexo, minus dense, sat distincte, utrimque fortius punctato; elytris subacervato-seriatim punctatis, sutura, margine inflexo posterius interstitiisque alternis subaeneo-nigris; antennis fere totis, apice femorum tarsisque nigris. Long. 8 — 10, Lat. $5\frac{1}{2}$ — 6 millim.

"Patria: Mexico (Mus. Holm., etc.); Nebraska et Texas (see Rogers, l. c.) Praecedentibus affinis. Ovalis, sat convexa, pallide sordide flava vel straminea, nitida. Caput subremote, distincte punctatum, lateribus baseos maculaque media triangulari, interdum etiam ore, nigris. Antennae basi flavo-testaceae, articulis quinque apicalibus clavam indistinctam formantibus, subtransversis. Prothorax elytris tertia parte angustior, lateribus parallelis, anterius angustatus, disco minus dense, sat distincte, utrimque dense et fortius punctatus, sat convexus, litura media ut littera V formata nec non maculis nonnullis lateralibus, plus minus confluentibus, nigris, ornatus. Scutellum laeve, nigro-limbatum. Elytra lateribus parallelis, subacervato-seriatim distincte punctata, serie punctorum prima paullo ultra quartam partem elytrorum extensa, margine inflexo posterius, sutura, vitta suturali anteriore angusta nec non utriusque vittis quinque interstitia alterna occupantibus, omnibus prope basin, intermediis tribus etiam prope apicem abbreviatis, subsuturali et marginali apice conjunctis, subaeneo-nigris. Pectus nigro-maculatum. Ventris segmenta macula utrimque laterali fasciaque abbreviata basali, interdum interrupta, nigris ornata. Pedes flavo-testacei, apice femorum tarsisque, interdum etiam macula femorum, tibiarumque basi et apice aeneo-nigris.

"♂ Segmento ventrali ultimo apice truncato."

Food, geographical distribution, habits, ecology, and juvenile stages exactly like the remainder of the species.

In life the elytral color is pale yellow-white, or rarely yellow in young, immature specimens. Its more narrow, anteriorly constricted pronotum, elongate form, and general color in live specimens give no suggestion of *decemlineata.* The reduced amount of fusion in the pronotal pattern, however, gives a condition closely similar to the northern species; it possesses, however, entirely different juvenile stages which are like *L. multitæniata* Stål. In collections, especially where data of localities is poor, it might easily be confused with the northern type. This "biotype" is identical in all respects, as here distinguished, with a specimen in the "Baily Coll." in the British Museum labeled "*multilineata* Stål," "Named by Stål," "Type Stål 176."

BIOTYPE L. MULTITÆNIATA-OBSCURA NOV. VAR.

This form occurs in nature, especially in the populations of *multitæniata,* in the valley of Mexico, and in the region of Puebla, often with considerable frequency, and is an extracted homozygous recessive of F_2 or F_3 that comes out in the crossing of the typical *multitæniata* and *melanothorax.* It has been the experience that the majority of the specimens found in nature were homozygous when isolated in cultures, and continued to maintain their type, but not infrequently individuals are found that are heterozygous, often of great complexity. The typical aspect of the pure-breeding recessive from nature is shown in figure 8, plate 14. I have seen it in nature only in that form, where it has the form and aspect of the modal *L. multitæniata* Stål, but with the head and pronotum entirely black, excepting for the edges of the pronotum, which are brownish or yellow, but with the pronotal markings entirely obscure. The ventral side and the legs are entirely black.

This form does not, as far as I am aware, maintain itself in continued genetic lines anywhere in nature, but is constantly breeding back into the general population. Its habits, juvenile stages, and ecological relations are in every respect like those of *L. multitæniata* Stål.

This form has been produced many times in crossing experiments between *multitæniata* and *melanothorax,* and as far as experience goes is always a recessive.

HABITUDINAL VARIETY INTERMEDIA NOV. VAR.

Leptinotarsa multitæniata-intermedia **nov. var. nov. sp., Tower, 1906,** *l. c.*

This form, a variety of *L. multitæniata* Stål, is found in the main in the north plateau of Mexico, and is distinguished from the southern variety by its smaller, less robust form, lower variability, conspicuous absence of minor divisions, uniform pale yellow-white color, and its freedom from extensive fusions of the elements of the color-pattern. It differs also in the larval stages, which are yellow, yellow-red, or pale red in color, with the same system of ornamentation, but more pronounced. As known from museum material, it might well be confused with the varieties of *multitæniata* or *decemlineata,* but the fact that it is a stable, genetically perpetuated type, distributed over a wide geographical range, and that it is alternative in its attributes to corresponding attributes of *multitæniata* in crosses, and its distribution leaves no choice but to class it as a habitudinal variety of the same species, or perhaps to raise it to specific rank. (Plate 4, fig. 9.)

DESCRIPTION OF LIVING ANIMALS.

Imago.—Above: Oval, convex, head and pronotum light yellow-brown, ornamented with black spots. Elytra pale yellow-white, with five longitudinal black stripes, second, third, and fourth often fused; edged with irregular double row of punctations, interspaces arched, polished; costal edge inflexed, grooved, smooth brownish or yellow-white. Head: Eyes black, mouthparts dark brown, anterior basal parts brownish, last six joints black, faintly pubescent, eleventh joint broader than long, twelfth conical, truncate, last six joints forming an elongated cone-shaped club. Epicranium faintly irregularly punctate, spots all present, variable. Pronotum smooth, polished, few faint punctures in posterior outer angle. Spots all present and not extensively fused, central **V**-spot $a' - \begin{matrix} am \\ + \\ pm \end{matrix} - a$ is a common condition, often also include $b' - b$; spots d', e', f', and

d, e, f, variable, but never fused to central system and never reaching to pronotum border; scutellum brownish black, polished. Below: Light yellow-brown, thoracic and abdominal segments with color-centers all distinct, rarely fused; legs: femora, ends black, often extending to near middle, which is brownish or reddish; tibia and femora yellow brown or yellow, ends black; tarsus brownish, legs smooth, polished, few minute punctures, and rarely minute spines.

Size: Male smaller than female, and both smaller than *L. multitæniata* Stål or *L. decemlineata.* Male, 7 to 11.5 mm. long, 5 to 6.5 mm. broad; female, 7 to 13 mm. long, 5 to 7.25 mm. broad.

Sexes: Female with sternal sclerite of last abdominal segment rounded smooth, and in male truncate and faintly grooved.

Food: Solanum rostratum or its allies, and *Solanum elæagnifolium* in the northern part of its range.

JUVENILE STAGES.

Eggs: As in *L. multitæniata,* but smaller; length 1.5 to 2.25 mm., 1 to 1.4 mm. broad. Incubation 6 to 10 days.

First larval stage: Head, pronotum, and legs black; body dull yellow color with sternal and pleural spots present, minute; tergal spots, especially outer and posterior members, reduced or wanting, variable. Length, 2.25 to 3 mm. at end of stage.

Second larval stage: Head, legs, and pronotum yellow-brown, latter with the posterior border black. Body dull yellow-red or ocher, spiracula spots strong; baso-pleural small, posterior members usually wanting or reduced to mere trace; tergal spots present only on thoracic and two front abdominal segments. Length, 8 to 12 mm. at end of stage.

Third larval stage: Like the preceding, but baso-pleural and tergal spots frequently almost gone. Body-color variable, dull yellow to dull yellow-red, never clear yellow of *L. mutitæniata* or clear wine-red of *L. decemlineata.* Length, 8 to 12 mm. at end of stage.

Pupa: Pupates in ground at depth 2 to 6 inches. Pupa like *multitæniata.* Pupal stage lasts 5 to 12 days, on average 9 or 10 days.

Length of ontogeny: Twenty to thirty-five days.

GEOGRAPHICAL DISTRIBUTION.

In 1906 I gave localities of occurrence. Further investigations have given a range indicated on plate 5, where it is seen to extend northward over the Mexican Plateau, up the Rio Grande Valley into New Mexico, and into Arizona towards the Colorado River. I have observed it in all its phases at the following locations: In the Rio Grande y Lerma Valley at Ocatlan, La Barca, Guadalajara, Zopopam, Encarnacion, State of Jalisco; in Rio Panuco Valley at San Bartolo, Rio Verde, Cardenas, San Luis Potosi, Espirito Santo, Matehuala, Vinegas, Catorce, State of San Luis Potosí; Monterey, Montemorelos, State of Nuevo Leon; Matamoros, Forlon, State of Tamaulipas; Monclova, San Pedro, Jimulco, State of Coahuila; Gomez Palacio, Durango, State of Durango; Aguas Calientes, State of Aguas Calientes; Rincon de Romos, Zacatecas, Fresnillo, State of Zacatecas; Ojito, Santa Barbara, Parral, Escalon, Jimenes, Sta. Rosalia, Chihuahua, Montezuma, State of Chihuahua; Tucson, Benson, Bowie in Arizona; Lordsburg, Rincon in New Mexico.

RECURRENT MUTANT L. MULTITÆNIATA-INTERMEDIA-MELANOTHORAX.

This recurrent mutant has only been observed at Octlan in Jalisco, San Bartolo in San Luis Potosi, and Sta. Rosalia in Chihuahua. It differs from the same mutant form in the southern portion of the range of *L. multitæniata,* by its smaller size and duller color of the head and puncture, which have little or no luster. I have not been able to find this mutant in New Mexico and have no knowledge of the relations of the *"melanothorax"* species found by Snow at Las Vegas. These, while undoubtedly this same mutant type, do not seem to be regular in occurrence in New Mexico, and in fact the mutant is rare over the entire range of the habitudinal variety *intermedia.* (Plate 12, fig. 7.)

HABITAT, ECOLOGY, AND LIFE HISTORY.

Like the preceding, this form passes the long, dry season (winter) in hibernation as an adult in the ground. It emerges over most of its geographic range when the summer rains begin, which is a variable climatic event. It breeds at once, producing progeny that grow rapidly and give adults in 3 to 5 weeks; these, over most of the range, after feeding for a few days may hibernate or breed again, giving a second generation which hibernates soon after emerging. Whether there be one or two generations per season is entirely dependent upon the climatic conditions of the locality. In localities like Tucson, where the summer rainy season may be short and not begin until August, one generation seems to be the rule, although I found two in 1909. On the plateau of Mexico, at Guadalupe, Zacatecas, two generations are the rule, although here there are exceptions. When brought to the laboratory two generations are the normal cycle, but the second one of these is easily suppressed by the use of desert complexes. Materials from nature are usually heterozygous with respect to the reproductive rhythm and must be purified before they are used in experiment.

Ecologically this form presents nothing different in principle or in the factors involved from *L. multitæniata* Stål, but differs considerably in minor details and relationships, especially in the strictly desert areas.

SOURCE OF MATERIAL.

Stock used for experimentation has been obtained at La Barca in Jalisco; San Luis Potosí, Catorce, Monclova in Coahuila; Jumilco, Zacatecas in Zacatecas; Chihuahua in Chihuahua, Mexico; Tucson in Arizona, and Deming in New Mexico, and has been fairly uniform in composition from all locations.

HABITUDINAL VARIETY MULTITÆNIATA-VARIABILIS NOV. VAR.

(Plate 4, figs. 10, 11.)

In the upper portion of the Rio Lerma Valley, especially on the plains at the foot of the Nevada de Toluca and eastward to the Sierra de las Cruces, occurs a geographically isolated portion of *L. multitæniata,* which differs therefrom in constant genetically reproduced characteristics, and even though no fast line of demarcation can be drawn, the gametic distinctness and the behavior of the restricted group leaves one with little choice as to its taxonomic place. I have

classed it as an habitudinal variety on account of its separation in space and its known differences of gametic constitution.

DESCRIPTION OF LIVING ANIMALS.

Imago: Broadly oval, convex, robust; in general, shorter and broader than *multitæniata,* color variable, orange-red or reddish yellow, pattern dense black; punctations weaker and surface more polished than *L. multitæniata.* Above: Head and pronotum reddish yellow, often orange or red, marked with dense, extensively fused pattern not variable, few scattered weak punctations on epicranium behind eyes and on lateral portion of pronotum; polished; on epicranium pattern center fused into general one which nearly covers entire part. Pronotum, central triangular spot reaching to anterior margin. $b'-a'-am-a-b$ with central lighter area; d', e', f' and d, e, f always fused and usually reaching posterior border in outer angle. Not infrequently fused with central area; c' and c always free. Mouthparts black or deep brown; antennæ black; basal joints sometimes brownish, terminal joint very short, conical; scutellum black, polished; elytra orange, yellow, red, variable, with 5 dense black longitudinal stripes, broader than in any other member of group, and edged with an irregular double row of feeble punctations. Second and third always fused partially. First and darkened anal margin form wide median band. Costal edge inflexed, smooth, flat. Below: Legs almost always black, tibia rarely brownish in central portion. Legs, polished, few punctures and minute scattered hairs. Thoracic and abdominal surface yellow-brown, with variable amount of fusion between color elements.

Size: Constant; male 10 to 13 mm. long, 7 to 9.5 mm. broad; female 10 to 14 mm. long, 7 to 10 mm. broad.

Sexes: Female with sternal sclerite of last abdominal segment rounded smooth and in male truncate, deeply grooved; female larger than male, but difference in size is less than in *multitæniata.*

Food: Solanum rostratum or allied species.

JUVENILE STAGES.

Eggs: Dark yellow or reddish yellow, laid on lower surface of leaves in bunches of 10 to 150, as in other forms; not often stalked, oval, ventrally concave, or flattened, smooth, polished. Length, 2 to 2.75 mm., 1.25 to 1.75 mm. broad. Incubation 6 to 12 days; average 8 days.

First larval stage: Head, pronotum, and legs deep shiny black; body reddish yellow or deep yellow; full complement of body-spots, each with one or more spines. Length at end of stage, 2.5 to 3.75 mm.

Second larval stage: Head, pronotum, and eyes shiny black; pronotum with anterior border deep reddish brown, brown, or rarely red; body-color variable, deep chrome-yellow, yellow-red, ocher; spiracula and basopleural spots present, strongly developed; few pairs of the inner tergals present at anterior end of the series. Length at end of stage, 4.5 to 6.5 mm.

Third larval stage: In color and pattern identical with second stage. Length at end of stage, 8.75 to 14 mm. Length of larval period varies from 15 to 25 days; averages about 18 days.

Pupa: Pupates in ground from 2 to 5 inches below surface; pupa yellow, with spiracula and variable tergal spots. Pupation lasts from 6 to 12 days; on the average 10 days.

Length of ontogeny: Twenty-one to thirty-seven days; average 25 to 30 days. The juvenile stages are variable in this geographical variety and some of the larval colors may be isolated by proper breeding into line-cultures, breeding true.

The range of this form has already been indicated and is shown on plate 5. It has been observed in all stages at the following points: Toluca, Lerma, Salazar, Ixtlahuaca, Malacatepec, Vale, Zitacuaro, Angangueo, Maravatio, in the States of Mexico and Michoacan.

RECURRENT MUTATION, MULTITÆNIATA-MELANOTHORAX STÅL.

Identical with same mutant of *L. multitæniata,* except that stripes are often broader upon the elytra.

Color biotypes are present and easily isolated by selective purification in cultures. These relate entirely to hypodermal color, and the following can be easily established: *L. variabilis* b^1, red elytral ground; *L. variabilis* b^2, orange elytral ground; *L. variabilis* b^3, yellow elytral ground; *L. variabilis* b^4, white elytral ground.

The life-history is like *L. multitæniata,* excepting that the growing-season is somewhat shorter and less favorable. Two generations per year are the general rule. The last generation may be seriously retarded or nearly annihilated by early autumn frosts, cold spells, or early advent of the dry season.

Ecology of this variety differs only in trivial details from the ecological relationship of *multitæniata,* excepting that it is in general compelled to meet more rigorous conditions than *L. multitæniata.*

Stocks for analysis and experiment have been obtained at the following points: (*a*) Toluca, from the open plain west of Toluca, especially on the northern side of Cerro de Zopilacalco and Cerro de Huitzila and from the banks of a small rainy-season pool east of El Temple de El Ranchito; (*b*) Lerma, on open plain to south of town on shore of small lake; (*c*) Malacatepec, 3 miles south of plaza, near an old ruined rancho; (*d*) Zitacuaro, near town, in waste places, especially in corrals or pastures.

LEPTINOTARSA OBLONGATA NOV. SP.

(Plate 4, fig. 12.)

Leptinotarsa oblongata Tower, 1906. Inv. Evol. Chrys. Beetles genus Lept., Carnegie Inst. Wash. Pub. No. 48, pp. 6, 20; Jacoby, as *L. decemlineata* Say, etc., Biol. Centr. Am., vol. VI, pt. 1, p. 233, and suppl., p. 255.

Jacoby refers specimens of this species from many Mexican localities to *L. decemlineata* Say, on account of "fulvous legs, more or less marked or spotted with black." The "fulvous" color of dead material is meaningless and useless as a diagnostic character, and the highly variable black markings are of service only when taken in conjunction with other characters. Some of the localities recorded by Jacoby refer undoubtedly to this form (Cuernavaca in Morelos); other localities are *L. multitæniata* Stål or its different geographical races. Jacoby's specimens now in the British Museum show clearly much confusion with respect to this species and *L. decemlineata* Say. I have been over this material and have given in tabular form below the determinations reached.

TABLE 1.

Jacoby's localities in Central America, from which specimens belonging to *oblongata* or *decemlineata* have been recorded as *L. decemlineata*.	Collector.	Jacoby's determination.	Determination made upon basis of present knowledge.	British Museum designations or "collection."
Mexico.				
La Parada	Sallè	L. 10-lineata	L. oblongata	Jacoby collection.
Do	Do.	L. multitæniata	L. oblongata	Do.
Toluca	Do.	L. 10-lineata	L. multitæniata hab. var. variabilis...	Do.
Do.	Do.	L. multitæniata		
Moncolva	Palmer	L. 10-lineata	L. multitæniata hab. var. intermedia..	Do.
Durango City	Höge	L. 10-lineata	L. multitæniata hab. var. intermedia..	Do.
Ventanas, Durango	Do.	L. 10-lineata	Probably a new species or var.; 5 specimens, all uniform.	Do.
Guadalajara, Jalisco	Do.	L. 10-lineata	L. multitæniata hab. var. intermedia..	Do.
Matamoros de Izuca	Do.	L. 10-lineata	L. oblongata	Do.
Cuernavaca	Do.	L. 10-lineata	L. oblongata	Do.
Tacambaro, Michoacan	Do.	L. 10-lineata	L. multitæniata hab. var. intermedia..	Do.
Vente de Zopilote, Guerrero	Smith	L. 10-lineata	L. oblongata	Do.
Mexico City	Höge	L. 10-lineata	L. multitæniata biotype multitæniata..	Do.
	Smith	L. multitæniata	L. multitæniata multitæniata	Do.
	Sallè			
Costa Rica.				
"Costa Rica"	Sallè (?)	L. 10-lineata	Is L. 10-lineata Say, as far as can be determined. No other specimens or records from Costa Rica. Is probably a mistake due to misplaced locality label.	British Museum collection.

Five specimens in the Jacoby collection in the British Museum, collected by Höge at Ventanas in Durango, Mexico, and recorded as *L. decemlineata* Say, are in all respects like *L. oblongata,* but of nearly double the size, with the same form and color-pattern system. I have never seen this type in nature, and do not know that *oblongata* is found that far north. They may represent another localized species of this group.

DESCRIPTION OF LIVING ANIMALS.

Imago: Elongate, elliptical in outline, convex, distinctly and constantly longer and narrower in proportions than any other species in the group. Above: Epicranium and pronotum yellow, rarely tan or yellow-brown, marked with black pattern. Epicranium rather regularly and uniformly punctate with medium-sized punctations. Pronotum evenly punctate on lateral portion and posterior border; less punctate in median anterior portion. Mouthparts dark yellow or yellow-brown, never black, eyes black; antennæ, basal joints yellow or yellow-brown, last six to eight black, faintly pubescent, sixth to eleventh broad as long, twelfth truncate, conical; epicranium pattern, anterior lateral epicranial spots fused, forming heart-shaped spot, lateral posterior epicranials free, distinct, reaching to posterior border of eyes, rarely fused in median line. Pronotum, color-pattern, spots a' and a parallel, united $a'-\frac{am}{pm}-a$, forming **U**-shaped spot; b' and b sometimes fused entirely to a' and a. d', e', f' and d, e, f groups often completely united, forming dense black spot in the posterior outer angle, and frequently fused anteriorly to b' and b. The modal condition of the pattern is expressed by the formula:

$$\begin{array}{cccccc} c' & b' & & +am+ & b & c \\ e'+d' & & a' & a & d+e & \\ +b & & +pm+ & & f+ & \end{array}$$

The pattern is constant and typical of this species; scutellum brownish, polished; elytra pale yellow-white typically, rarely yellow or red, with anal border black and five longitudinal black stripes; second and third always united posteriorly, sometimes also fourth; all edged with irregular row of punctations. Costal edge inflexed, flat or slightly concave, smooth, yellow, shoulder reduced. Below: Yellow or yellow-brown, spots all present, variable amount of fusion; legs, yellow or yellow brown, rarely red, femora mostly black or deep brown, tibia ends black, tarsus black, femora sparingly punctate.

Size: Smaller than *multitæniata* or *decemlineata,* constant. Female larger than male.

Sexes: Female with sternal sclerite of last abdominal segment rounded complete, smooth, and in male truncate, grooved, extending to near middle of plate; variable in male; female also more robust than male and broader posteriorly.

Food: Solanum rostratum or near relations, but will eat *S. tuberosum, S. elæaginofolium,* and *S. hertwigi* when other food is denied it, but they do not thrive thereon and soon die out.

JUVENILE STAGES.

(Tower, 1906, pl. 19, figs. 13, 14, 15.)

Eggs: Pale yellow, oval, smooth, polished, laid in small bunches of 5 to 50, on lower surface of leaves, rarely stalked; ventral side flattened and slightly

concave. Length, 2 to 2.75 mm.; breadth, 1 to 1.35 mm. Incubation lasts 5 to 10 days; average 7 days.

First larval stage: Head, pronotum, and legs, black; body bright yellow, spiracula spots only present. Length at end of stage, 2.5 to 3.25 mm.

Second larval stage: Exactly like first stage in all respects, except that anterior edge of the pronotum may be brownish or yellow in variable degrees. Length at end of stage, 3 to 5 mm.

Third larval stage: Like second, except that pronotum always has anterior portion yellow. Body color deep yellow. Length at end of stage, 9 to 14 mm. Length of larval life from 12 to 25 days; average 15 days.

Pupa: Pupates in the ground at a depth of from 1 to 5 inches. Pupa yellow, spiracula spots present. Pupation lasts from 8 to 16 days; average 10 days.

Length of ontogeny: 25 to 46 days; average 32 to 35 days.

GEOGRAPHICAL DISTRIBUTION.

As far as information is available, this form is strictly limited to the Rio Balsas Valley below the Mexican escarpment on the west, the Oaxaca-Guerrero Highlands, and southward to the Isthmus of Tehauntepec (plate 5). I have seen it in all its stages many times at the following locations: Cuernavaca, Yautepec, Jojutla, Triente, Puente de Ixtla in the State of Morelos; Iguala, Naranjo, Los Amates, Balsas, State of Guerrero; Cholula, Atlixco, Tatetla, Matamoros de Izucas, Tlancualpican, State of Puebla; Oaxaca, Mitla, Tlacolula, Tula, San Juan, El Parian, Tecomavaca, Venta Salada, State of Oaxaca (Tower, 1906, p. 7). The records of Guadalupe and Texcoco, valley of Mexico, are of *L. multitæniata,* biotype of *L. multitæniata* Stål. Added localities: Mescala, Venta, Zopilote, Chilpancingo, Chilapa, Tetela de Rio, State of Guerrero; Ocotlan, Ejutla, State of Oaxaca, extend its known range well over the Oaxaca-Guerrero Highlands, but I have no records from the Pacific slope, and I have not found it as far south as the Isthmus of Tehauntepec, nor north beyond the Rio Tepalcatepec Valley.

HABITAT, ECOLOGY, LIFE HISTORY.

Living everywhere in regions having a long, hard, dry season and a short, sharp, rainy season, two general periods occur—one of quiescence passed as an adult in hibernation in cemented cells in the earth and the other in active growth and reproduction. The beetles emerge from hibernation at the end of May or early in June, begin breeding by the middle of June, and produce a generation which emerges by the latter part of July, which again breeds at once and gives a second generation, which emerges at the end of August or early in September. The generation soon enters hibernation and remains dormant until the following May. At Cuernavaca and Oaxaca this cycle is very regular, although there is frequent overlapping and intercrossing of broods at all locations. Materials from nature are, therefore, very frequently impure in respect to the reproductive rhythm, and must be purified before utilization in experiments.

SOURCE OF MATERIAL.

The materials for experimentation in this species have come from the following localities, with the exception of a few unimportant strains: (*a*) Cuernavaca: Stock No. 617, from Rancho de Basoco, to east of town, on a loma sepa-

rated by two deep barrancas from the rest of the valley; stock No. 619, from sides of deep barranca between Cuernavaca and San Anton; stock No. 621, from the top of the loma between the barrancas north of trail from El Hacienda to Tetlama. (*b*) Atlixco: Stock No. 615, from cultivated fields 2 kilometers to south of town. (*c*) Oaxaca: Stock No. 607, from foot of Maote Albans, on rolling, well-drained area; stock No. 605, from north and west sides of Cerro del Fortin, to northwest of Oaxaca City.

BIOTYPES.

Four biotypes are capable of being developed from some strains of this species, especially those from the northern and eastern edge of its range: *L. oblongata* b^1, red elytral ground-color; *L. oblongata* b^2, orange elytral ground-color; *L. oblongata* b^3, yellow elytral ground-color; *L. oblongata* b^4, white or pale yellow-white elytral ground-color. These are especially prone to develop in material from that portion of the habitat along the south flank of the Nevada de Toluca, but I have not been able to develop them from materials obtained in the Oaxaca-Guerrero Highlands.

LEPTINOTARSA RUBICUNDA NOV. SP.

(Plate 4, fig. 7.)

Leptinotarsa rubicunda, Tower, 1906. Inv. Evol. Chrys. Beetles, genus Lept.; Carnegie Inst. Wash. Pub. No. 48, pp. 7, 21.

The history of this form as I have found it leaves me greatly in doubt as to what status it should have in this taxonomic orientation. When first found in 1903 it occupied a considerable range on the plain between Toluca and the Nevada de Toluca, and the Sierra de Las Cruces, covering an area estimated at 10 to 12 miles square. It differed constantly in all stages from *L. multitæniata-variabilis*, and was not observed inter-breeding with it at any time. In cultures it is genetically constant and true to type indefinitely, and it genetically perpetuates itself in nature in the same constant manner. In 1906 I was able to find it only in a limited area of about half an acre in extent near Mexicalicingo, and in 1908 only 30 specimens could be found in a week's search at the height of the season, and in 1909 and 1910 no trace of the form could be found. The last members of the race were as constant and were in all respects like the first found, and while never abundant at any time, it was fairly easy to discover 20 to 50 in a day's search, and it was obtained in the earlier years in considerable numbers. Its rapid decline and apparent extinction raise some interesting questions.

Because of its genetic distinctness and constant differences from the obviously near relatives I have retained it here as a species. Future information may change this orientation.

DESCRIPTION OF LIVING ANIMALS.

Imago: Broad, rounded, convex, dark red or crimson, with heavy, shiny black color pattern. Above: Epicranium, pronotum, and elytra uniform deep red or crimson, never orange or yellow; epicranium evenly punctate with distinct pits, anterior lateral epicranial spots fused and reaching anterior border, rarely prolonged caudad; lateral posterior epicranial spots forming band on posterior portion and anteriorly to eyes; eyes black; mouthparts black, polished,

pubescent; antennæ, basal joints reddish, remainder black, pubescent, sixth to eleventh broad as long, twelfth truncate, conical, broader than long. Pronotum minutely, evenly, sparingly punctate; few larger scattered punctations near lateral margin, polished. Pattern constant, central widely open V composed of

$$\begin{array}{ccc} c' \quad b'+ & & +b+c \\ & a+am+a & \\ & + & \\ e'+d'+ & pm & +d+e \\ +b'+ & & +b+ \end{array}$$

in which elements are entirely and constantly obscured in all examples. Spots *d', e', f'* and *d, e, f* always fused and reaching posterior border, and usually fused to central area, all by *d'*-anterior end of *a'* and *d*-anterior end of *a*. Central area frequently reaching border of pronotum. Elytra: Anal border shiny black with 5 broad, shiny, black longitudinal stripes; third and fourth usually fused posteriorly, all edged with irregular row of rather small punctations, arrangement variable, in some specimens only single row, in others some rows are partly double; in none are they developed to the extent found in *multitæniata* or its varieties. Elytra polished, costal edge inflexed, red, smooth, flat, polished, never punctate. Below: Red body-color, nearly covered by color-pattern composed of fused elements to the extent that usually only edges of sclerites are red; legs, all shiny black, slightly pubescent femora and tibia punctate, often strongly.

Size: About same as *multitæniata* but less variable, and sexes of nearly equal size. Male, 9 to 12.25 mm. long, 7.5 to 9 mm. broad; female, 9 to 13.5 mm. long, 7.5 to 10 mm. broad.

Sexes: Female with sternal sclerite of last abdominal segment complete, rounded, smooth; male truncate, grooved; female also broader posteriorly than male.

Food: Solanum rostratum, S. tuberosum (wild stock) on Nevada de Toluca.

JUVENILE STAGES.

(Tower, 1906, plate 17, figs. 16, 17, 18.)

Eggs: Bright red, large oval, polished; 3 to 3.5 mm. long, 1.25 to 1.75 mm. broad; ventrally flattened and concave, laid in bunches on lower side of leaves, 10 to 50 in a bunch, never stalked. Incubation lasts 8 to 12 days; averages 10 days.

First larval stage: Head, pronotum, and legs shiny black; body bright red with full set of color markings. Length at end of stage, 3 to 4.5 mm.

Second larval stage: Head, pronotum, and legs, shiny black; body bright red, spiracula, baso-pleural, and the anterior two or three pairs of inner tergal spots present. Length at end of stage, 5 to 7.5 mm.

Third larval stage: Exactly like the second stage, excepting that pronotum has the anterior edge red and the anterior tergals wanting. Length at end of stage, 10 to 17 mm. Length of larval period, 12 to 20 days; average 15 days.

Pupa: Pupates in ground, 2 to 4 inches below surface. Pupa red, spiracula and tergal spots present, variable. Pupation lasts from 8 to 17 days; averages 10 to 11 days.

Length of ontogeny: 28 to 50 days; averages about 36 days.

GEOGRAPHICAL DISTRIBUTION.

Northern slope of Nevada de Toluca below oak zone, 8,500 to 9,500 feet; in open grasslands. Recorded from following locations in all its stages. (Plate 5.)

HABITAT, ECOLOGY, AND LIFE HISTORY.

As far as known, the life-history of this species is like that of *L. multitæniata* Stål. It passes the long, dry, cold winter in the imago stage, hibernating in the ground, emerges at beginning of rainy season in June, breeds by the middle of June, and produces a first summer generation which emerges about the end of July. Then these breed again and give a second generation, which emerges late in August or early in September. This second generation was always observed to be small and was several times apparently absent. In cultures a normal rhythm of two generations in each reproductive cycle was found.

SOURCE OF MATERIAL.

The stock for breeding and testing came from the following locations: (*a*) Toluca, on open plain to south of city, about 1 kilometer south of Capilla de San Sebastian; (*b*) Mexicalicingo, on open plain near town.

LEPTINOTARSA DECEMLINEATA SAY.

(Plate 6, fig. 5; plate 7, fig. 2.)

Doryphora 10-lineata Say, 1824. Journ. Acad. Nat. Sci. Phila., III, pt. 2, p. 453; Rogers, 1857, Proc. Acad. Nat. Sci. Phila., VIII, p. 302; Suffr., 1858, Ent. Zool., XIX, p. 244.2; Walsh, 1865, Pract. Ent., I, No. 1; Harold, 1874, Berlin Zeit., p. 444.

Leptinotarsa decemlineata Gemminger et Harold, 1874. Catalog. Coleop., t. IX, p. 1, p. 3440; Jacoby, 1883. Biol., Centr. Am., vol. VI, pt. 1, p. 233; pl. XIII, fig. 24, suppl., p. 253.

And many other authors. No doubt is attached to the determination of this form.

Say's original description is as follows:

"Yellow; thorax litterate, with black; elytra each with five black lines. Inhabits Missouri and Kansas. Body yellow; head with a triangular, black, frontal spot; thorax two abbreviated black lines, divergent before; about six black dots on each side; elytra, sutures, and five lines on each, black; the interior line is confluent with the suture behind; exterior line marginal; three intermediate ones joined or approximated at tip; beneath, incisures and three or four series of ventral spots black.

"Length two-fifths of an inch.

"Var. a elytra white; the two outer, intermediate lines are united at base and tip.

"This species seems to be not uncommon on the upper Missouri where it was obtained by Mr. Nuttall and myself. The variety I found on the Arkansas."

DESCRIPTION OF LIVING ANIMALS.

Imago.—Above: Oval, convex, robust, variable in form, size, and color, depending upon age, geographic location, and conditions of growth during ontogeny; head, epicranium, and pronotum yellowish brown, rarely reddish, in sexually mature specimens, often reddish in freshly emerged specimens, with variable pattern of black spots; epicranium rather uniformly punctate with moderately developed pits, most numerous behind eyes, eyes black, mouthparts yellow brown, rarely black; mandibles black, tips of palpi black, basal joints brownish; antennæ, basal 5 or 6 joints brownish, last 6 broadened, broad as long, black, slightly pubescent, last joint short, conical, often nearly obscured.

Pattern elements all present but variable. Pronotum rather coarsely punctate, often strongly so on lateral and posterior portions, center more freely punctate, polished. Pattern: All elements present but highly variable, from mere trace to extensive fusions. *a'* and *a* always divergent, forming V more or less open. Scutellum yellow-brown, smooth, polished, edges darkened. Elytra, in sexually mature specimens, pale yellow or straw-color, often more yellow or even yellow-red, variable with locality, food and age; anal border black, 5 longitudinal stripes, edges with irregular double row of moderate punctations, second stripe often united to third and these in turn to fourth stripe posteriorly; interspaces not raised, polished, costal edge inflexed, flat, or slightly concave, smooth, polished, yellow or brownish; hind wings strong, well developed, transparent, reddish or bright red in center, darker yellow or brown on costa and at base, variable. Below: Yellow-brown with pattern elements all present, variable, often much fusion; thoracic and abdominal surfaces smooth, polished, rarely, faintly punctate; legs, femora and tibia yellow brown or reddish, rarely black, joints darker brown or black, femora smooth, polished, rarely and freely punctate; tibia, few irregular, variable, well-marked punctures and spines.

Size: Female variable; male, 7 to 16.5 mm. long, 4.5 to 9 mm. broad; female, 8 to 12.5 mm. long, 5.5 to 10.25 mm. broad.

Sexes: Female with sternal sclerite of last abdominal segment rounded complete; male with same sclerite truncate, faintly notched, rarely grooved. Female larger than male, more rounded and broader behind; male more elongate in form and often very small.

Food: S. tuberosum, S. rostratum, and more rarely many species of *Solanum.*

JUVENILE STAGES.

(Tower, 1906, plate 17, figs. 19, 20, 21.)

Eggs: Laid in bunches, 5 to 200, not often stalked, oval, polished, smooth, concave on ventral side; length 1.75 to 2.50 mm., breadth 0.75 to 1.1 mm. Incubation, 5 to 15 days; average 6 or 7 days.

First larval stage: Head, pronotum, and legs, black; body red with full set of color markings, often spined. Length at end of stage, 3 to 4.5 mm.

Second larval stage: Head and legs, black; pronotum, posterior edge black, anterior portion red, reddish yellow, or brown; body red, varying in tint with the condition of the animal, food, climatic conditions, etc., baso-pleural and few anterior inner tergals present. Length at end of stage, 5 to 6.75 mm.

Third larval stage: Like second stage, color variable; length at end of stage, 11 to 17 mm. (Plate 12, fig. 2.)

Length of larval life, from 10 to 28 days; average between 13 and 15 days, but much depends upon food and climatic conditions.

Pupa: Pupates in ground, from 1 to 5 inches below surface; pupa reddish yellow. Pupation lasts from 6 to 15 days, but averages 8 to 10 days.

Length of ontogeny: 21 to 58 days; averages between 28 and 31 days.

GEOGRAPHICAL DISTRIBUTION.

(Plate 5.)

North America, eastward from the foot of the Rocky Mountains to the Atlantic coast, northward into Canada, and northeast throughout the St. Lawrence Valley, southward to the Gulf coast and to lower Texas. Common in most localities; is everywhere a grassland form and becomes a serious agricultural pest in many areas, especially in the north and east. (See Tower, 1906, for further account of distribution and migration.)

HABITAT, ECOLOGY, LIFE HISTORY.

Like the other species in this division *L. decemlineata* passes the winter as an imago, hibernating, but there is a great variation over the area inhabited in the dates of entrance into and emergence from hibernation, due almost entirely to varying climatological conditions. On emergence it breeds, giving the first summer generation, which emerges about the middle of July over most of its range, and these breed again, giving the second summer generation, which emerges in August or early September and soon hibernates. It is not uncommon to find a third small brood, especially in the south, and in favorable seasons in other places, and this due to the fact that in nature the overwintering population may live and breed with the second summer generation, producing hybrids between generations of different potentialities. The progeny of these matings often numerous and strong, behave in a very curious and variable manner, so that in materials in nature, two, three, or even four broods are found at times. When, however, stocks from many different portions of the country are fully analyzed, it is found that all, both north and south, are two-brooded like the rest of the species in this division.

MINOR DIVISIONS.

Many years of breeding and experimentation have shown that an endless number of true-breeding races of low variability can be created in experiment. None of these are well defined, or of any independence in group-cultures or in nature, and none has any homologue in nature. All are due to purely artificial isolations and fixings of trivial variations of attributes present, and while an imposing array of pure biotypic lines could be created, it would be the acme of folly to do so, because they have no discoverable permanence in the species in nature.

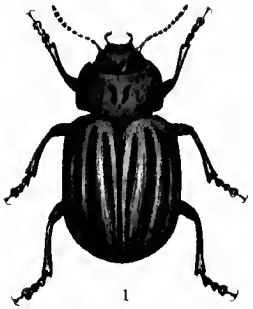
Sports are known sparingly in nature. In 1906 I recorded the occurrence of several which were then regarded as such, some of which proved on breeding to be gametic variations, and it is only by breeding that the real nature of these variations can be determined. It is not uncommon to find somatogenic variations, some of which closely resemble the germinal variations. Breeding tests alone can determine the true nature of these extreme variations.

SPORTS OBSERVED IN NATURE AND TESTED BY BREEDING.

LEPTINOTARSA PALLIDA NOV. VAR.

(Plate 6, fig. 2.)

Imago: Oval, convex, more elongate than *decemlineata* Say, white or faintly yellow-white; smaller than *L. decemlineata* Say, with sides more nearly parallel. Above: Epicranium sparingly, irregularly, and not deeply punctate, densest behind eyes, center polished, few punctations, color pale white with tinge of yellow, pattern extremely reduced, but center usually variable; eyes black, mouthparts pale; mandibles, tips brown or black; antennæ, four basal joints white, remainder very light brown, faintly pubescent, seventh sharply broadened, broad as long, as are also eighth to eleventh, twelfth conical, rounded. Pronotum, few small scattered punctations on lateral and posterior margin, whole part polished; pattern reduced, elements all present, but fusions are rare. Pronotum, distinctly longer and narrower than in *L. decemlineata* Say; scutellum smooth, polished, brown, edges darkened; elytra white or pale yellow-



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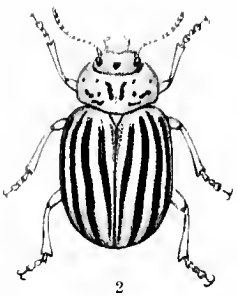

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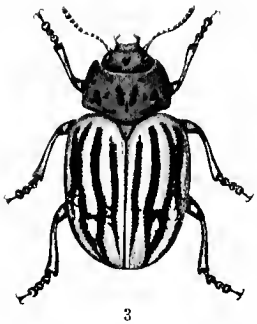

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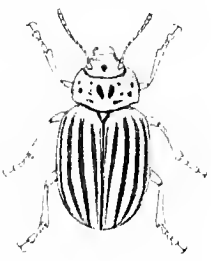

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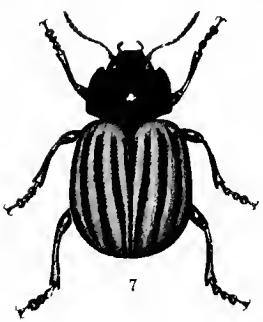

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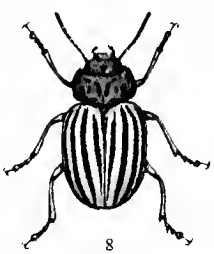

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9

K. TODA DEL.

JULIUS BIEN, N. Y.

TYPES OF MODIFICATIONS PRODUCED IN EARLIER EXPERIMENTS FROM L. DECEMLINEATA.

1. Form *rubrivittata*. × 2.5.
2. Form *pallida*. × 3.
3. Form *tortuosa*. × 2.5.
4. Form *melanicum*. × 3.
5. *L. decemlineata*. Normal stock form used. × 3.
6. Form *albida*. × 2.5.
7. Form found by Snow in upper part of Rio Grande Valley, near Las Vegas, and called *L. melanothorax*. I have found it once at Las Vegas on *S. eleagnifolium*—not the same as *L. melanothorax*. Figured from specimen in Kansas Museum. × 3.
8. Form *minuta*. × 3.5.
9. Form *defectopunctata*. × 3.

white, anal edge brown, never black, 5 longitudinal dark bands, fifth always brown, remainder always dark brown, never black, all edged with irregular double row of punctations. Interspaces flat, while elytra have frequently granulated appearance due to numerous fine furrows which often radiate from the punctations. Shoulder more depressed than in *L. decemlineata,* costal edge inflexed, smooth, flat, white; hind wings always white or transparent, no color present. Below: White or whitish yellow, color-centers often wanting entirely upon thorax and abdomen, which are smooth and polished. Legs: Light joints browned, tarsus brown, femora sparingly punctate, first pair more so than other, tibia faintly punctate, small spines.

Size: Rather constant, body index, male 7 to 12.5 mm. long, 4.5 to 8 mm. broad; female, 7 to 13 mm. long, 5 to 8.5 mm. broad.

Sexes: As in *L. decemlineata* Say.

Occurrence: In nature near Cold Spring Harbor, Long Island, New York, 1899, tested, breed true; Cabin John Bridge, Maryland, 1900, not tested fully, some breed true; in F^2, Clifton, Ohio, 1901, 6 tested, breed true; Chicago, 1902, 2 tested, breed true. (Tower, 1906.)

Juvenile stages: As in *L. decemlineata* Say, but uniformly lighter in color and lower development of color elements.

LEPTINOTARSA MELANICUM NOV. VAR.

(Plate 6, fig. 4.)

Imago: Rounded, convex, robust, shoulder prominent, raised, dark ocher-yellow with dense black, much-fused color-pattern. Above: Epicranium, mouthparts, antennæ, eyes, dense shiny black; antennæ, seventh to eleventh joints nearly as broad as long, pubescent, terminal joint conical, longer than broad; epicranium strongly punctate; pronotum rather regularly and densely punctate, least so in center; ocher-yellow with pattern having all elements fused and much enlarged; scutellum black, polished; elytra, dark ocher-yellow, anal border black, 5 longitudinal black stripes, second, third, and fourth always united posteriorly, costal edge inflexed, smooth, flat, black or brownish anteriorly, shoulder prominent, all elytral punctations strong. Below: Head black, thoracic and abdominal segments quite or nearly so; all color-pattern elements fused, body-color ocher-yellow; legs entirely black, femora and tibia strongly punctate.

Size: Larger and more robust than the average *L. decemlineata* Say and much broader in proportion to length; not variable. Male, 12 to 16 mm. long, 7 to 10 mm. broad; female, 12 to 16.5 mm. long, 8 to 10.25 mm. broad.

Sexes: As in *L. decemlineata* Say, but male is more strongly marked.

Occurrence: In nature: West Bridgewater, Massachusetts, 1895, 2 tested, breed true; Cold Spring Harbor, Long Island, New York, 1899, 1, not completely tested, indications were that it would breed true; Cabin John Bridge, Maryland, 1900, 29, not tested.

Food: As in *L. decemlineata* Say.

Juvenile stages: As in *L. decemlineata* except that body-color of larvæ is always dense red, verging towards a brownish tinge.

LEPTINOTARSA RUBRIVITTATA NOV. VAR.

(Plate 6, fig. 1.)

Imago: In all respects like *L. decemlineata* Say, but characterized by the bright-red hypodermal color of the head, pronotum, elytra, and ventral surface;

breeds true; color is often almost a scarlet in freshly emerged specimens, but changes to full red at maturity.

Size: As in *L. decemlineata.*

Sexes: As in *L. decemlineata.*

Occurrence: In nature: Stock from McPherson, Kansas, 1903, gave in 1904 at Chicago 1 male able to transmit character. Liberal, Kansas, 1905, 1 male, 2 females, breed true.

Food: As in *L. decemlineata.*

Juvenile stages: As in *L. decemlineata.*

LEPTINOTARSA TORTUOSA NOV. VAR.

(Plate 6, fig. 3.)

Imago: In form, color, sculpture, and pattern, except elytral pattern, like *L. decemlineata;* elytral pattern, anal edge black, five longitudinal black stripes fused into a variable network of many transverse fusions. Median stripe shortened, much so posteriorly, not reaching more than two-thirds length of elytron, bent anteriorly at region of middle band and unites with ramous stripe which is bent posteriorly and fuses with inner subcostal stripe distally; subcostal approximated and much anteriorly, and other subcostal bent at middle base sharply forward to meet costal stripe which is shortened and often not well developed; punctations much reduced and consist of a single irregular row along edge of each stripe; interspaces flat, polished, smooth.

Size: As in *L. decemlineata.*

Sexes: As in *L. decemlineata.*

Food: As in *L. decemlineata.*

Juvenile stages: As in *L. decemlineata.*

LEPTINOTARSA ALBIDA NOV. VAR.

(Plate 6, fig. 6.)

Imago: Elliptical, elongate, convex, smaller than *L. pallida,* white with light-brown pattern. Above: head, pronotum, and elytra white, with color-center present, greatly reduced, and all light brown in color; elytral stripes shortened and narrowed, punctations on head and pronotum about like those of *L. pallida;* antennæ, sixth to eleventh joint broader than long, terminal joint short, rounded, almost hidden; elytral punctations, single irregular row. Below, like *pallida.*

Size: Smaller than *decemlineata.* Male, 7 to 10.5 mm. long, 4 to 6.5 mm. broad; female, 7 to 11.25 mm. long, 4 to 7 mm. broad.

Sexes: As in *L. decemlineata.*

Occurrence: San Antonio, Texas, 1904, 1, tested, breeds true—*i. e.,* was able to transmit its characters *in toto.*

Food: As in *L. decemlineata.*

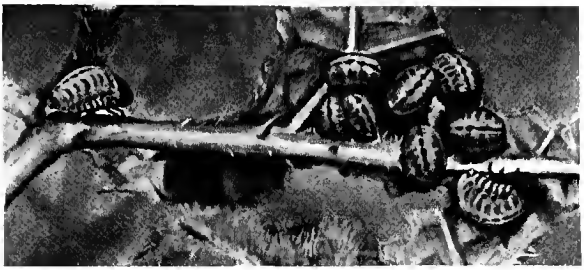
Juvenile stages: As in *L. decemlineata,* only smaller larvæ and body-color pale red or pink.

LEPTINOTARSA MINUTA NOV. VAR.

Imago: Body-form, proportion, color-pattern, and sculpture like *L. decemlineata,* excepting that *L. minuta* is always less than half the average size and frequently only one-third that of *L. decemlineata.* (Plate 6, fig. 8.)

Size: Body index, male, 5 to 9.5 mm. long, 4 to 5.25 mm. broad; female, 5 to 10.25 mm. long, 4.5 to 6 mm. broad.

Sexes: As in *L. decemlineata.*



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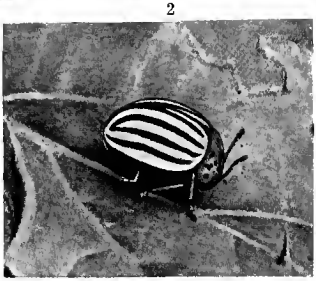

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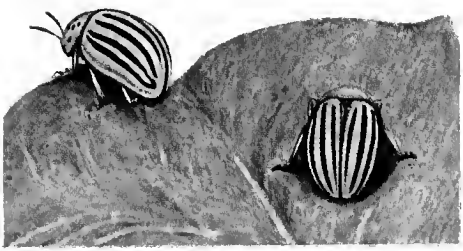

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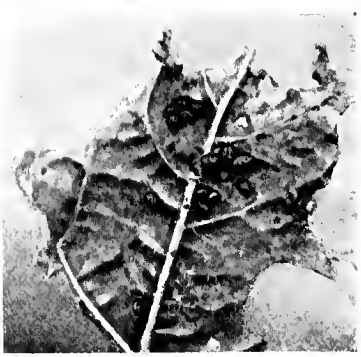


9

K. TODA DEL. JULIUS BIEN, N. Y.

1. *L. juncta.* Larvæ, 3d stage. × 1.
2. *L. decemlineata.* Larvæ, 3d stage. × 1.
3. *L. juncta.* From life. × 1.5.
4, 5. *L. decemlineata.* From life. × 1.5.
6. *L. texana.* Larva, 3d stage. × 2.
7. *L. tumamoca* n. sp. Larva, 3d stage. × 2.
8. *L. tumamoca* n. sp. From life. × 2.
9. *L. signaticollis.* Larvæ, from life: (*a*) 1st stage; (*b*) 2d stage; (*c*) 3d stage. × 1.
10. *L. signaticollis.* Larvæ, from life: (*a*) 1st stage; (*b*) 2d stage; (*c*) 3d stage.

Occurrence: In nature: Cabin John Bridge, Maryland, 1, not tested; Yellow Springs, Ohio, 1901, 1, tested, bred true; Chicago, Illinois, 1902, 1, tested, bred true; San Antonio, Texas, 1904, not tested (Tower, 1906).

Food: As in *L. decemlineata.*

Juvenile stages: As in *decemlineata,* excepting that larvæ in second and third larval stages are always very small in size.

Ecology: Elytral stripes edged with single regular rows of impressed punctations. Often depressed and placed at the bottom of a groove.

JUNCTA DIVISION.

LEPTINOTARSA JUNCTA GUER.

(Plate 7, fig. 3.)

Chrysomela juncta Guer., 1824. Ins. spec. nov., pp. 590, 825. Stål, 1862, Monog. Chrys. d. l'Am., pt. 1, p. 165; Kraatz, 1874, Berl. Zeit., p. 442, 1, fig. 6.

Doryphora juncta Rogers, 1857, Proc. Acad. Nat. Sci. Phila., VIII, p. 30, 1; Suffrian, 1858, Ent. Zeit., XIX, p. 243, 1.

Leptinotarsa juncta Guer. Gemminger et Harold, 1874, Catalog. Coleop., t. XI, pt. 1, p. 3440.

This distinct and easily recognizable species is clearly separated from all of the foregoing forms by the character of the elytral punctations first noted, I believe, by Walsh (Am. Ent., vol. I). The original type I have not seen, but Rogers's and Stål's determinations leave no doubt of the correctness of the identification. Stål's description, taken from his monograph, is quoted merely for completeness in taxonomy:

"Ovalis, flavo-testacea, antennis apicem versus nigro-fuscis; maculis capitis, prothoracis ventrisque nec non scutello nigris; elytris stramineis, striato-punctatis, interstitiis alternis nigris. Long. 9½–11, Lat. 6–7½ millim.

"Patria: America borealis, Georgia. (Mus. Holm., etc.)

"Ovalis vel subovata, flavo-testacea, nitida. Caput apicem versus distincte punctatum, maculis tribus vel quinque parvis, una basali, duabus mediis, duabus prope apicem, nigris. Antennæ apicem versus sensim nonnihil incrassatæ, articulis apicalibus infuscatis, latitudine paullo longioribus. Prothorax elytris nonnihil angustior, antrorsum sensim subangustatus, anterius utrimque leviter rotundatus, obsolete parce punctulatus, utrimque punctis raris distinctis instructus, angulis anticis acutiusculis, vittulis duabus discoidalibus abbreviatis nec non maculis pluribus parvis nigris ornatus. Scutellum læve. Elytra lateribus parallelis vel retrorsum leviter ampliata, distincte striato-punctato, seriebus punctorum basin haud attingentibus, quarta et quinta, septima et octavo longe ab apice, sexta et nona prope apicem coëuntibus, interstitiis secundo, quarto, sexto et octavo nec non dimidio interiore interstitii decimi nigris, interstitio septimo reliquis angustiore, interdum fusco-testaceo vel nigricante. Venter maculis, in series transversas dispositis, nigris ornatus. Femora subtus interdum macula nigra ornata.

"♂ Segmento ventrali ultimo apice truncato."

DESCRIPTION OF LIVING ANIMALS.

Imago: Stål's description of this distinct form leaves only comments as to color necessary. In life the elytral ground-color is ivory-white and the head, pronotum, and ventral side is light yellow-brown, making it one of the most beautiful members of the genus. Much variation exists in the color-pattern,

and between the type and variety (*a*) as described by Stål all kinds of intergrades occur. (Plate 7, fig. 3.)

Size: Larger and more robust than *L. decemlineata;* sexes of nearly equal size. Male, 11 to 16 mm. long, 8 to 11 mm. broad; female, 11 to 17.5 mm. long, 8 to 11.75 mm. broad.

Sexes: Female with sternal sclerite of last abdominal segment rounded; male with the same sclerite truncate and faintly grooved.

Food: Mainly *Solanum carolinensis.* More rarely egg-plant or other succulent or herbaceous Solanaceæ. Experience in cultures shows that *S. carolinensis* is the only food upon which it thrives, and while it will eat other plants (*S. esculentum, S. tuberosum*), it in my experience does not thrive upon them, and can not be reared for more than one or two generations thereon.

JUVENILE STAGES.

Eggs: Large, oval, rarely stalked, polished, smooth, pale yellow-pink in color; 2 to 3 mm. long, 1 to 1.5 mm. broad; ventral flattening very slight. Incubation, 7 to 12 days.

First larval stage: Head, pronotum, and legs, black or dark brown; body, pale bluish-pink, variable, spots variable, spiracula always present. Length at end of stage, 3 to 4.5 mm. (Plate 7, fig. 9.)

Second larval stage: Head, pronotum, legs, as above; but the dark color on the pronotum is limited to the posterior edge and is variable; body, pale bluish-pink, spiracula spots only present. Length at end of stage, 5 to 7 mm.

Third larval stage: Exactly like second (see Walsh, Am. Ent., I). Length at end of stage, 10 to 16 mm. (plate 7, fig. 1). Length of larval life, 12 to 30 days; average, 20 days.

Pupa: Pupates in ground at depth of 1 to 5 inches. Pupa yellowish. Duration of pupation 10 to 25 days; average, 14 days.

Length of ontogeny, 30 to 60 days; average, about 40 days.

GEOGRAPHICAL DISTRIBUTION.

(Plate 3.)

Irregularly and sparingly distributed over Atlantic Coastal Plain and nearby areas. Not often common, and considerably restricted and reduced in numbers and range since the advent of *L. decemlineata.* Does not extend southward into Mexico, as far as has been discovered.

Material for experimentation has come from the following locations: Atlanta, Georgia; Mobile, Alabama; Lexington, Kentucky, and New Orleans, Louisiana.

GEOGRAPHICAL VARIETY TEXANA SCH.

(Plate 7, fig. 5.)

Much confusion exists concerning this not uncommon form from Texas and the Rio Grande Valley. It has been variously classed as *L. defecta* Stål, as Say's variety of *decemlineata,* while Townsend has described its larvæ as those of *L. undecimlineata.*

I have reared it for many generations and find that it is genetically true and constant to its type when grown side by side with *L. juncta* under the same conditions and on the same food-plant—*i. e., S. carolinensis.* It differs from *L. juncta* Guer. in a few minor attributes and these only in degree, not in kind. I have, therefore, classed it as a geographic variety, it being comparable to the other similar varieties here recognized. In that I have no means of knowing

whether Say found this form on the Arkansas or not, I have only included his possible notation because it is often so included in taxonomy.

Doryphora decemlineata Say, 1824. Journ. Acad. Nat. Sci. Phila., III, pt. 2, p. 453.

Doryphora defecta Stål. Specimens identified by Horn and others are found in many museums and collections, and while closely like Stål's *defecta*, both are distinct, as I can testify from having both alive at the same time to compare.

Leptinotarsa decemlineata Say var. *texana* Schaeffer, 1906. Bull. Brooklyn Inst. Arts, Sci.. I. No. p. p. 239.

Schaeffer's description is as follows:

"*Leptinotarsa decemlineata* var. *texana*, new variety. Form and size of *decemlineata* but differs in having the punctures of the striæ regularly placed, not confused. The first black elytral vitta abbreviated at about the middle, the submarginal entirely absent and the epipleuræ entirely pale, from base to apex.

"Brownsville, Texas.

"I have given this form a name, which seems to be quite constant in southern and southwestern Texas, as it stands in several collections as *defecta* Stål, from which it differs in several points. In *defecta* the suture is always black, the third and fifth intervals are only slightly wider than others, very distinctly and much more in var. *texana*, the inner margin of elytral epipleuræ and apex is always black and all the markings on elytra have a very distinct æneous tint, which is never the case in *decemlineata* and var. *texana*, and is twice mentioned in Stål's description. The specimens of *defecta*, which I have taken from Brownsville, Texas, have the suture, the sixth elytral interval, from apex to not quite to base and the eighth from base to not quite to middle blackish-æneous; the eighth interval is in some specimens at apex also darker, as well as the fourth, which is then more or less connected with the vitta on the sixth interval, the sutural interval being counted as the first."

Schaeffer is entirely correct in distinguishing the form from Stål's *L. defecta* and correct in his notation of the elytral punctation, a character that at once places it as allied to *L. juncta* and not *L. decemlineata*. When reared, the juvenile stages are unmistakably like *juncta* and not like *decemlineata*.

DESCRIPTION OF LIVING ANIMALS.

(Plate 7, fig. 5.)

Imago: I would add to Schaeffer's description as follows: Color in life, head, pronotum, and elytral ground-color pale yellow, often almost white. Pronotal color-pattern variable in size of spots, but fusions between them are rare.

Size: Constant.

Sexes: Female with sternal sclerite of last abdominal segment rounded flat; male with same sclerite truncate, grooved.

Food: Solanum carolinensis, S. elæagnifolium.

JUVENILE STAGES.

Eggs: Exactly like *L. juncta*, only paler; 2.5 to 3 mm. long, 1 to 1.5 mm. broad. Incubation lasts 5 to 12 days; average 7 or 8 days.

First larval stage: Exactly like *L. juncta*, only paler. Length at end of stage, 3 to 4 mm.

Second larval stage: In all respects like *L. juncta*, but color is paler. Length at end of stage, 5 to 7 mm.

Third larval stage: Like *L. juncta,* smaller, paler (see Townsend, Trans. Tex. Acad. Soc., vol. 5, pp. 51–101). Length at end of stage, 8 to 14 mm. (Plate 7, fig. 6.)

Length of larval life: 15 to 30 days; average 22 days.

Pupa: Pupates 1 to 3 inches below surface of ground. Pupa pale yellow. Pupation lasts 10 to 20 days; average 14 days.

Length of ontogeny: 30 to 60 days; average 50 days.

GEOGRAPHICAL DISTRIBUTION.

Rio Grande Valley in Texas and northern Mexico; possibly northward into the valleys of Red and Arkansas Rivers. I have one specimen labeled Pueblo, and to this has been added Colorado, but I have no confidence in the correctness of the locality. Accurate records: Dallas, Brownsville (Sch.), Laredo, Corpus Christi, Fort Worth, Santa Tomas, San Antonio, Texas; Matamoros, Rosita, Ciudad, Porfirio Diaz, Mexico.

Habitat, ecology, life-history: In cultures this form has always given two generations per year—one early in the season, then a long wait and one late in the season, then hibernation through the winter. In nature I suspect that this is also its behavior, but I have not had opportunity to fully verify this point. It is also probably variable in behavior in nature.

LEPTINOTARSA TUMAMOCA NOV. SP.

DESCRIPTION OF LIVING ANIMALS.

(Plate 7, fig. 8.)

Imago: In shape, general color, and pattern much like *L. texana,* but only half the size. Above: Oval, convex, epicranium and pronotum light yellow-brown marked with black; epicranium strongly and densely punctate on sides and posterior portions, center polished, few small or no punctations present. Pronotum densely punctate on lateral portions; center and posterior margins smooth, often polished; eyes black, pattern on epicranium lacking, or anterior lateral epicranial spots showing only a trace thereof; pronotal pattern, *f'* and *f* almost always absent, as are *am* and *pm, a'* and *a* never fused, but *b'* and *b* almost always fused with *a'* and *a;* scutellum dark brown, polished; elytra, ivory-white or yellowish ivory-white; anal edge yellowed or brown (cubital and anal stripes lacking), medials present, well developed; ramous and anterior subcostal always united posteriorly and posterior subcostal often united to anterior subcostal at or anterior to its junction with the ramous; costal stripe bent, shortened posteriorly, all edged with regular single row of punctations. Costal edge inflexed, smooth, flat, yellow. Below: All parts uniform pale yellow, edges of sclerites and joints sometimes brownish, antennæ and terminal joints slightly darker, no pattern on thorax or abdominal segments.

Size: Smallest member of group and possibly of the genus; is not larger and probably averages smaller than *L. lineolata.*

Sexes: Female, sternal sclerite of last abdominal segment rounded, flat, smooth; male with same sclerite truncate, slightly grooved. Male slightly broader and larger than female.

JUVENILE STAGES.

Eggs: Small, oval, ends squared, not stalked, laid on lower surface of leaves singly or in small bunches of 10 to 20; pale bluish-yellow or yellow; 1.25 to 2 mm. long, 0.6 to 1 mm. broad. Incubation lasts from 5 to 10 days; average 6 or 7 days.

First larval stage: Head, pronotum, and legs dark brown, or rarely black; body pale lavender or yellowish, spiracula spots alone present, small. Length at end of stage, 2.5 to 3.5 mm.

Second larval stage: Exactly like first. Length at end of stage, 3 to 4.5 mm.

Third larval stage (plate 7, fig. 7): Strongly resemble small larvæ of *L. texana,* color variable, pale dilute lavender to pale bluish-yellow; pronotum and head often brown or yellow-brown; legs variable, brown to black. Length at end of stage, 5 to 8.5 mm. Length of larval life, 10 to 18 days; average about 14 days.

Pupa: Pupates in ground from 0.5 to 2 inches below the surface. Pupa yellow-white; pupation lasts 6 to 12 days; average 8 days.

Length of ontogeny: 25 to 40 days; average about 30 days.

Geographical Distribution: Limited to desert areas of Arizona and Sonora. Recorded from Hermosville, Guaymas, and Nogales, State of Sonora, Mexico; Benson, Tucson, and Maricopa, Arizona.

Habitat, ecology, life-history: Apparently there is but one generation per year of this form. This has been our experience at Tucson and in cultures. Hibernates as an imago.

Source of material: Bottoms of Santa Cruz River in moist midsummer at foot of Tumamoc Hill, Tucson, Arizona.

EVOLUTION AND PHYLOGENY OF THE LINEATA GROUP.

The present conditions in any group or organisms in nature afford little basis for creating schemes of descent, and where fossil evidence is lacking the usual substitute of ontogenetic stages, similarities, distribution phenomena, and the like usually result in expressing the bias of the student rather than relationships. To many this procedure is proper and correct, and many believe it is still able to give accurate pictures of descent. When applied to large groups and longer trends of evolutionary development this may in a very crude manner be partly true, but for minor groups and for the building of schemes of relationships within genera the method can hardly be considered as safe. Former methods and consideration in phylogeny were based upon the unproven assumption that descent had always been by dichotomy, either by slow divergent variations and extermination of intermediates or by a saltation, both a dichotomy of existing materials, and so grew up the familiar tree-like schemes of descent, but to what extent these represent the truth no one at present can honestly decide.

The accumulated experience of the neo-Mendelian investigations shows beyond doubt that attributes and qualities can be and are shifted about, replaced, and recombined, giving quite different expressions to the new combinations. As a result nothing new appears; only an organic metathesis occurs, which, nevertheless, produces distinct, divergent, individual entities. To what extent are the grouped lines of descent which we call species the product on a larger scale of metathesis in nature? Who can answer? The Linnæan view that crossing is indicative of and synonymous with degeneration in standard and stamina in the species is hardly tenable at this time, and in the attempt to arrive at some comprehension of the probable past history one can not forget the part that crossing may have played in conjunction with other processes in the formation of the evolved group.

The inertia of taxonomy and orthodox biology seems to be yielding to the opinion that species in nature are not all due to dichotomy and extermination, but that intermingling or crossing is a potent factor in species formation, and increasingly frequent are the expressions of opinion that species may after all be hybrids (Morgan) and that frequent intercrossing has occurred (Cook).

In the *lineata* group of the genus *Leptinotarsa,* fossil evidence is lacking, and I have no confidence in ontogentic sequence of characters as a basis for determining relationship.

The data of distribution also are subject to valid criticism as a basis for phylogeny, less valid than ontogeny; but too much reliance can not be placed upon distribution data. Nor can habitat and adaptability be of any considerable value, because the physiological capacities which make for habitat selection and ecological restrictions are as fully capable, and are transferred by metatheses as often as are purely morphological characters.

The *lineata* group is limited to North America; beyond doubt the *undecimlineata* division is strictly tropical grassland in distribution and ecological relations. The *multitæniata* division, on the other hand, is always a temperate grassland form, either on the high plateaus of the tropics which have a temperate climate or on the plains farther north; at any rate, it in the main is absent from the areas occupied by the first division. The *juncta* division, in its range, gives one the impression of being decadent and limited to areas of peculiar complex like the coastal plains and subtropical deserts—areas of high temperature and considerable humidity for a short growing season, then a long period of drought and quiescence. These are observed conditions in nature where the divisions present more than distributional and ecological distinctions, each having marked morphological characters, and between them intercrossing is not only not common, but is difficult in most instances to obtain.

In each of the three divisions of the group there is an undefinable impression of a fundamental basis, a form nucleus in each upon which the array of shifting characteristics is placed; whether this is a reality or only a product of the combined characteristics present opinions would differ, and all would be merely opinion, not demonstration. Within each division, however, the arrangement of the characteristics gives species, which cross and show metatheses of their attributes in the products of crossing.

I could, of course, create a phylogeny for these materials, but it would be necessary to assume certain premises as true which could not be tested, and too much of this sort of biology has already been created. In their earlier history this general group, the Chrysomelinæ, reaches far back towards the base of the Mesozoic era, and there the genera show species distinguished by much the same characteristics as distinguish species and genera to-day. Since the rise of the Chrysomelinæ no one has any conception of the myriad of species and lesser divisions that have come and gone in their history, and yet there persists through all a basis of form, and the array of attributes and qualities capable of metathetic shifting and recombination, giving species and varieties. The time has passed when schemes of phylogeny can be of interest and current assumptions of monophylitic origin and dichotomy, either slow or rapid, are vanishing into meaningless metaphysics.

That there are long-continued lines of descent no one doubts; and perhaps all of our trends of evolution and also phyla are but syntheses of factors from

diverse sources, producing stable and pliable aggregations of qualities which are incessantly worked over, modified, recombined, and adjusted to newer conditions from time to time, giving a trend of evolution and a multitude of species.

For us nature contains only other organic forms diverse in kind, intricate in action, and all of most vital interest to us, since we are one of them and have come into being through the same processes that gave rise to them. Phylogeny and its schemes all too often lead to a static condition of mind and to orthodoxy in biology. There exists only living matter with its qualities manifest in diverse combinations. These species, genera, etc., of our scheme of cataloging, lasting though they may be, are but expressions of the operation of the general processes of germinal distintegration, synthetic recombination, and the rise of new factors in the germinal complex, all interacting in an amazingly complex series of operations. These are the centers of present inquiry because they are the *modus operandi* of evolution. As for schemes of past evolution in these materials, I have none and have no basis for building one. I know which species are most alike and which are most different, but likeness does not always mean nearness of kin, nor unlikeness remoteness of genetic relationship. They are to me only so many kinds of living substance, each with its proper and restricted qualities, attributes, and conditions, which I am able to use, like reagents in a test-tube, for many operations of analysis and synthesis, and thereby help, I hope, to add to our knowledge of the fundamental processes that are productive of heterogeneity and evolution in organisms.

CHAPTER III.

THE PROBLEMS OF GAMETIC CONSTITUTION.

INTRODUCTION.

Knowledge of the gametic constitution of organisms has been one of the desert spots in biology, because there has been, up to recent times, no adequate working hypothesis for use in investigation, with the result that there has been endless interpretation and profitless speculation that has served more to confuse than to advance the analysis of the gametic constitution and the behavior of characters in genetic lines of descent. Darwin's provisional hypothesis of pangenesis, and the subsequent conceptions that were the natural outgrowth of it, lead only to controversy, interpretation, and plausibilities, and were not capable of use as working hypotheses in experimentation. The Galtonian hypothesis stated only the results of a statistical blending of the data and arrived at no analytical results, nor can the hypothesis be utilized in the orientation of any critical experimental analysis. The rediscovered work of Mendel and the neo-Mendelian developments that have taken place in the past decade, especially the advances made by Cuèrrot, have produced, at the least, a useful working hypothesis.

In the course of this investigation I have had abundant opportunity to test the two current hypotheses, without prejudice to either. The inherent defects in the Galtonian hypothesis were early apparent; the early dogmatic assertions with regard to the Mendelian principles, the rabid partisanship of many of its adherents, unfortunately gave it the setting of a cult, and led, naturally, to suspicion of its principles. Both of the hypotheses were put to severe test through the course of this investigation.

The net result of the severe tests was the complete failure of the Galtonian hypothesis in all respects and at every point and the demonstration that the principles of Mendel were true in their broader aspects when stripped from the encumbering conceptions of " fixed units " and Weismannism. The conceptions of the separateness of manifestation of "characters" as the result of the interaction of specific agents in the germinal material, the capacity of these for transfer or metathesis, and the random metathetic distribution of characters into different classes of gametes, were put to every sort of test that I could devise, but in no instance, thus far, have I been able to satisfy myself that tests made have in any manner been at variance with the real principles, and, in short, have in every respect throughout confirmed them and in some directions carried the investigation farther than friendly investigations in its support would have done. In the effort to find characters that " did not Mendelize," meaning the fact that they were not definite in manifestation and were not able to " segregate " but blended in crossing, I have had no success. In these cases on further testing I have always found either conditions in the medium, in the organism, or associations of the character with others that, when worked out, showed entire agreement with the principles of factorial constitution and operation.

In this second section of this report I shall present such facts concerning the behavior of different characters, and of the gametic agents productive thereof as have a bearing upon the other portions of the investigation, and also some of the data of the attempts to analyze the gametic constitution of my materials. In this I have used simple characters of structure, color, and complex associations thereof, and simple reaction characters and complex associations thereof.

The problems of heredity for the present, in experimental investigations, center entirely about the methods of attack that arose from the rediscovery of Mendel's work and as formulated in the present neo-Mendelian conceptions. At the basis lies the idea of the individuality of manifestation and production of "characters," and out of this has grown the misconception that the "characters" are separate entities or individualized in the organism. It is distinctly not true that the characters are so individualized, excepting in our recognition of them as end-results or products of the operations of interacting antecedent agents, and even in the observed product our methods of perception and description tend to an excessive symbolic delimitation of them that is not justified or representative of the truth. If it can be kept clearly in mind that the characters in organisms are the same as in non-organized substances, the resultant of the combining, interacting materials or factors of composition and the conditions of the medium at the time of combination, no difficulty is introduced into the problems of gametic constitution by the pragmatic individualization of characters in plants and animals.

With this conception of characters in organisms is associated the idea of the antecedent gametic factors of composition whose interaction and presence in the germinal material, the interactions and products of which must be provided for by proper conditions in the medium. This idea of the factor or agent that makes possible, and the determiner, the agent that of several possibilities because of its presence decided the direction of the resultant reaction, are most decidedly not "biological" conceptions, but are in all respects a purely physical conception of the problem, and precisely the same as exist in non-organized substances. Neither factor or determiner nor any other agent is the carrier of anything, any more than water of crystallization is the carrier of the crystalline form or color or hardness. Determiners and factors need not even be substances, but may well be, in many instances, physical positions and relations in the parts of the gametic substances. Many instances in non-organized substances could be cited where this single fact of configuration of the system was a determiner of the action of the substance, and produced differences in the reaction and in the products because of this initial difference of physical position and arrangement of the parts of the system, and there is no *a priori* reason why the same may not be true of organisms.

The fact of the segregation or distribution during gametic genesis of these agents into different classes of gametes does most to disrupt cherished traditions, and some of the conceptions and discussions that have been put forward by neo-Mendelians, attempting to give a particulate shifting and sorting in the gametes, is on the face of it absurd, and no better than Weismannism. In every mating there are numerous contrasting pairs of equivalent characters, but why some cross from one gametic system into the other, and others do not, and what determines which shall undergo metathesis, is entirely unknown. In one series

of crosses that I had there were 41 contrasting characters that were known to be alternative and that had been proven to undergo metathesis in experiment. This number of characters would, on the pure particulate basis, demand the formation in F_2 of thousands of classes of gametes and an impossible number of combinations thereof. No such extensive metatheses were found, nor have been found, in any organism that I know of, but there is a firm association of these agents in the gametes, and only a few show metatheses in any one combination, and this is most important. The association of the agents, and the determination of their position and relation in the system of the gametic mass, receive much-needed impetus from the recent developments in the study of sex-limited and other characters in *Drosophila* by Morgan, and gives the first published, clearly investigated data, upon this most important aspect of the problem.

No portion of the entire subject is of so great import as is this point concerning the position and relation of these agents in the system of the organic mass, and the relation of these to the possible dissociations and recombinations that may be produced during gametogenesis, and the number of kinds of gametes that are produced. This is the basic problem in the investigation of the gametic constitution of organisms in the future, and has to do with the analysis of the composition of the system of the organism, the position and relations of the agents, and the capacities for the dissociation, recombination, and replacement under standard conditions, and also under divergent constellations of environic factors. There is no reasonable doubt but that there are dissociation, recombination, replacement, and that in gametogenesis there are different factorial combinations produced in the gametes, such that when in combination in the zygotes they produce in one or more "characters" unlike results. That there is the random sorting of all the agents in gametogenesis, giving chance distributions of all of the agents and equal numbers of all possible kinds of gametes, is so highly improbable that it is not worth any consideration at all.

It is true that there is something acting in the production of gametes of different kinds that gives wonderfully consistent and precisely repeated results, and no doubt there is at the basis of the operations a series of phenomena with which we are dealing in accurate operations, but of which our commonly expressed conceptions are, no doubt, far from the truth. The mere fact that the verbal description of the reactions and results has been crude, particulate, vitalistic, or otherwise objectionable lies entirely in the wording and formulation of the operations as we have tried to think of the mechanism "in biological terms," and not at all in the operations or results—a condition that is not limited to this field alone. One needs but recall the first formulations of the agents operative in the field of immunity for an example of a professedly untrue picturing of the mechanism and of the agents, but with no question of the existence of the agents and the certainty and positiveness of the resultant reactions.

One of the most influential conceptions introduced by Mendel was the demonstration that the union of the gametes in fertilization was purely a chance one, and that the resulting combinations or classes of zygotes produced followed closely the expected array that results from the random union of the number of classes of gametes present in equal numbers. It gave little chance for "selective fertilization" and for "selection" or "choice" of any sort, pur-

poseful or otherwise. As far as investigation has gone all instances show that the combinations are purely chance, and although the use of "selective fertilization" has recently been utilized in "explaining" some results of crossing, it is not as a demonstration but as interpretation. It is safe to utilize for working the hypothesis that all fertilization is chance and await the demonstration of the instances of selective fertilization and the discovery of the actual mechanism of the process.

One aspect of the fertilization problem that has not received the attention that it deserves is the rôle of environic conditions to which the uniting gametes are subjected in the production of changes in the operations of the zygote, and even in the non-operation of some combinations. In this there is no selection, only the physical inhibiting of action or development by conditions in the medium, which, if the conditions persist, result in the elimination of the combination, solely because it can not operate, and so ceases to exist. This is a vital portion of the problem that has as yet been too little studied and may have much to do in determining dominance and in other ways modify the action of the combinations produced by the union of unlike gametes. The work of Tennant on echinoderm fertilizations and my modifications of the behavior by means of external conditions indicate some of the possibilities in this field. Further data upon this same topic is presented in the following pages. It should be considered as a possible agent in the investigation of "irregular" results in the products of crossing, and it is not at all improbable that the condition of the somatic tissues and fluids surrounding the gametes of internally fertilized organisms might well play an important rôle in the decision of the direction and character of the ensuing processes.

It is not unlikely that the further knowledge penetrates into these processes the more frequently will the play of the conditions of the medium about the point of reaction become of recognized importance, and this may involve the entire organism in its medium, or the portion, or character in the system of the organism, in which the surrounding portions and the external medium become, in combination, the important and efficient agents in determinations of the resultant reaction and its products. In evolution there seems, in the organisms I have used, the best of reasons for utilizing this idea as one of the means of attack upon otherwise difficult problems, and one that promises some measure of profit.

While one can not overestimate nor fail to admire the work of Mendel and its influence upon the developments of the past decade, all too often its broader implications have been lost in the desire that has apparently actuated many to make of it a cult, "Mendelism," as something apart from the rest of related phenomena, and attempts are made to attribute to it many things not possible from the very nature of its fundamental conceptions and means of operation. Some adherents have, seemingly, at least tried to make of it a highly special and permanent "force" in nature. This is most unfortunate. Mendel developed a viewpoint, a concept of the nature of characters, a method of analysis of the problems of constitution of the organism, and of the phenomena of heredity, and out of these the neo-Mendelians, especially Cuèrrot, have developed extensions of the ideas of Mendel. Our understanding of the nature of the agents will change from time to time; their terminology may alter, and the discovery

and isolation of actual agents well extend our knowledge and conceptions far beyond that of the present, but at the base there is a method of approach to the problems that has permanence of usefulness and philosophical value which will insure its retention in the methods of investigation. With this some may not agree, but the past decade has moved far beyond the knowledge and insight of the problems that Mendel had; but in all, the method of investigation, the determination of the relations of the factors of composition to the products, or characters, and the dynamic, mechanistic conception of these most fundamental operations of organisms, in terms of the interaction of the combining materials, are in essence the same as the formulation of the same problems of constitution in the non-living world.

The problems of heredity as investigated by Mendelian methods lead directly into some of the most fundamental portions of the general problems of the constitution of living bodies, the system of organization, their development, and transmutation in evolution.

THE GERM-PLASM CONCEPT.

The collective experiences of the last quarter century, and especially the concepts that have been the natural outgrowth of the writings of Weismann, have forced upon us concepts from which there is no present escape, namely, of the existence of gametic material that bridges the gap between generations that are constant in composition and organization, highly conservative with regard to changes, which persist without alteration throughout long-continued genetic lines of descent. Moreover, in allied lines of descent there are basal similarities, with alteration of portions of the system, that give species, genera, and all of the groupings of natural living things into the taxonomic divisions, into which we are by custom prone to classify the objects of our study.

From many fields of investigation—phylogeny, evolution, experimental embryology, genetics, and experimental evolution—consistent and mutually confirmatory evidence supports the concept that this genetic material, the germ-plasm, is not only remarkably stable in its composition and in its arrangement into a physical system of action, but also that all changes of an evolutionary character first occur in this material, by some alteration in its composition, whereby the action thereof is altered in respect to some character or characters. If therefore, any conceptions of the mechanism of evolution are to be had from experimental investigations, it is necessary, first of all, that some exact knowledge of the gametic system itself be had before much can be done in the attempt to modify it experimentally. In other words, it is necessary to understand something of the nature and composition of the essential materials with which we are working.

In the attempts to determine this we are unfortunately compelled to deal entirely with indirect analysis, in which the composition and structure of the genetic substance is described in terms of its results, as observed in the soma, and from these results project experiences backward into the germ-plasm, forming such idealized concepts of its factors of composition and of its structure as our perceptual experiences with the soma seem to justify. Indirect and unsatisfactory as this method is, the methods now in use enable one to derive lines of descent with stability of organization, and to repeat series of events, or to

produce desired combinations and results that are well within the expectancy of error, thus showing that the method has at least some present value in determining the nature of this substance, its factors of composition, its structure, and exact mode of action in specific combinations.

The conception of exact conditioning agents in the germ-plasm, so logically and philosophically developed in Weismann's writings, and the proof of these that came through the advances made after the recovery of the investigations of Mendel by the neo-Mendelian investigators, does not leave any doubt of the existence in the germ-plasm of agents of diverse potentialities, whose presence is necessary for the production of specific end-results in the soma.

The investigations of the neo-Mendelians show in an incontrovertible manner that there are gametic agents whose action can be predicted with entire mathematical accuracy; but it has been asserted that these agents concern only superficial and unimportant characteristics in the organisms investigated, and that there is a basis that is not at all dissociated to which these agents are attached as more or less unimportant additions.

It is undeniably true that the great majority of the characters of any one parent entering into a mating not only retain their identity, but also their association with the total complex of characteristics that came from that parent, and that only one or at most only a few are lost or transferred or replaced as the result of the crossing. There is a retention of the specific parental gametic complex of the species. Does this indicate a different material basis, or only the retention of the integrity of composition and structure of the system, with capacity to act for a time in union with some other system, but no ability to enter into a permanent union therewith?

In the crossing of natural species it has been my experience that the species base retains its integrity with exactness, but with varying degrees from complete integrity, giving only exact and typical monohybrid reactions and the extraction of pure parent types in F_2 through series in which one or two or a few characteristics were interchanged, giving new synthetic extracted types in subsequent generations to complex cases in which there was much interchange, and lastly to most intricate examples in which as far as could be determined new and permanent combination had been made between the two uniting gametes, with loss of many portions of the two parental systems, giving rise to new combinations that were, as far as could be determined, permanent in character and action. In these series of experiences, details of which are given in the chapters that follow, I have not been able to draw any line of demarcation, on one side of which is the species base and on the other the superficial gametic agents that are capable of interchange or loss. As far as my experiences go, it seems the most logical concept that if the factorial working of these basic phenomena of organisms are true in part they are also true in all characteristics of the organism, and that the germ-plasm must be thought of as a physical system with exact composition and structure, precisely as in the non-living materials of the planet, with differing degrees of capacity for dissociation and metathesis of factors. This capacity for dissociation and metathesis is not alone due to the nature of the specific form, but also to the reactions and the conditions of reaction to which the complex is subjected. That is, in one set of reactions under one set of conditions, there may be complete retention of integrity, as in

the cross of *L. signaticollis* and *L. diversa,* giving a simple monohybrid reaction, with no interchange of characters, while both species in other combinations show interchange of characteristics, and *L. diversa* and *L. decemlineata* form at the other extreme a fixed combination, composed of elements from both parents which it has not been possible to dissociate.

It therefore appears to me that capacity for dissociation is not necessarily a property of the basal species complex, but rather due to the reactions to which the parental gametic masses are subjected, both in the way of the combination in which they are united, and also depending upon the conditions of the medium under which the union or reaction is carried out. In this there is no difference in principle from the union of non-living materials where the nature of the reaction is entirely a product of the combination made, and the conditions under which the reaction is set to operate.

This capacity of the two gametic systems to unite for the production of a heterozygote, acting in harmony for the production of a soma, with two parental groups of potentialities which attain a balance for the space of a single generation, but with no permanent capacity for union, with the total separation of the parental systems in gametogenesis, presents no end of fascinating problems for the embryologist and geneticist, but concerning which there is at present entire lack of knowledge.

THE GAMETIC AGENTS.

Since we must admit the existence of specific gametic agents, or factors of composition, and since there is entire probability that the total sum of characteristics of the organisms are conditioned by the factors of composition arranged in a system, what about the agents themselves? Are they discrete, invariable units, present or absent in their totality, or do these agents represent centers of activity, foci, of greater or less magnitude? The Weismannian concept of fixed units was adopted bodily by the neo-Mendelians, and much the same idea is retained by De Vries in his mutation theory; but the fixity of these units seems to be a constant cause of trouble to Mendelian workers; hence the concept of multiple allelomorphs and kindred concepts developed by Bateson and others, all with the purpose of retaining the concept of the fixed, quantitative unit factor as a static morphological element of structure.

All of our ideas upon this point are based upon our perceptual experiences with the soma; certain body-characters appear or are absent in their totality in a given cross; it follows, therefore, that each is due to a factor which we symbolize as *A* or *a,* and unless further investigated each is asserted to be a unit factor, an entity. In some other combination, *A* is shown to break up into two or more portions, and it is therefore a compound allelomorph; but where is the limit of this compounding? In the first experience the action was a unit or center of activity, while in the second experience a hitherto single center of action, divided into two, each acting independently as far as the end-results are concerned; but what evidence is there from these experiences that we have reached the limit of divisibility? Further, what logical basis is there in this perceptual experience for the assertion that individualized entities have been discovered or isolated in the operations, or shifted therein? Conclusions as to the unit nature of the agents in the gametic material productive of these observed results in the soma are based upon the *a priori* assumption that organ-

isms are composed of ultimate "biological units of structure" which must be thought of in the same manner as Weismann conceived of his biophores. Any such idea is essentially vitalistic, for the reason that the unit is in all respects a living individual, and between it and the non-living there must be a sharp division, or else we must think of more remote agents of composition, and so on without limit.

On the other hand, if we think of the observed results produced in the soma merely as products of gametic reaction centers of a purely physical nature, no difficulty is introduced into the interpretation of the observations, because there are in physical phenomena ample evidences of the fragmentation of reaction foci into two or more discontinuous and independently acting centers, which may be permanent or transient, depending entirely upon the surrounding materials and conditions in the medium.

The basis of the ideas with regard to the fixity of these ultimate units of structure and activity was derived first from the interpretation of nature and evolution phenomena, and later by the neo-Mendelian experiences in crossing, in which morphological considerations dominated the logic and philosophy of the writers, developing dogmatic definitions and concepts of the discrete, discontinuous nature of both agents and of somatic characters. The neo-Mendelian observations showing the alternative transfer of characteristics is no criterion for the deduction that the agents are discrete and exist as units. It is convenient to symbolize them for purposes of description, but it must not be forgotten that it is only a symbolization and nothing more. In organisms we are not dealing with static masses, but with highly complicated integrations of material, in which endless physical and chemical reactions are in ceaseless operation. In this mass there are innumerable centers of activity at all times, in the germ as in the soma, and as the result of experiences with the materials that I have had to study, I can only regard the gametic agents as expressions of centers of activity of greater or less magnitude, bound into a system of operation which in the main retains its totality of manifestation throughout successive generations; but this retention of uniform aspect is not so much the product of its structure and composition as it is that it has in its successive generation cycles the same kind of materials to react with, and also essentially the same conditions in the medium in which to carry on these reactions; therefore repetition of reactions and results must naturally follow.

The fact of the perceptual experience of the metathesis of characters in crossing seems to me no evidence of the transfer of fixed units of any kind. The same gametic system, *L. signaticollis,* in one combination acts as a unit, in another combination it acts as two, or three, or more, depending upon the losses or replacements in its minor portions. Moreover, in my materials I have not been able to decide where the lower limit of unit manifestation was to be drawn in any instance. For example, in the complex pronotal pattern it may behave in crossing as an entity and may be symbolized as a unit of activity, or specific and trivial elements of it may show this same capacity for metathetic action, giving typical Mendelian reactions, and discontinuity of the somatic manifestations, and stable extracted products, in F_2, and in no instance have I been able to satisfy myself from experimental evidence that I had attained the limit, present in the materials for the production of this alternative, discontinuous type of

activity in process and products. I see no escape from the conclusion that there are not fixed and discontinuous units in the gametes, but rather that the results that we observe in the soma, and with which we are able to deal in accurate analytical and synthetic operations, are after all nothing more than reaction systems of greater or less magnitude, capable of endless fragmentation, depending upon the nature of the materials and the conditions of the medium at the time of combination. Each of these portions of the system as we observe its somatic manifestations is symbolized for purposes of description, but is no warrant for the concept of the existence of final indivisible units of any sort, nor for discontinuity between them. In the material composition of the gametes there can be no other discontinuity or isolation of the factors of composition than that which exists in any physical substance or in a complex mixture of substances.

At present there is no evidence as to the nature of any gametic agent, and it is quite possible that factorial differences may be due not only to actual differences in component materials, but also to differences in molecular arrangement or to changes in reaction directions or in velocity. In fact, there are so many possible modes of the production of factorial differences in materials that are as complex as the living substance of the gametes that it is hardly worth consideration at the present.

The location of the different agents in the gamete is, and is likely to remain, a question of debate for a long time. Cytological considerations and argument have for many years placed all of the "bearers of the heredity characters" in the chromatin, or at least in the nucleus, but there seems to be increasing evidence, especially in the field of experimental embryology, that the cytoplasmic portion of the gamete, especially of the egg, is also the bearer of hereditary characters. These relate especially to form and symmetry, or to kindred deep-seated phyletic qualities of the species.

Depending upon the nature of one's perceptual experiences with these problems of organic phenomena and the initial philosophical viewpoint hinges the concept of the structure of the germinal material that we create.

METATHESIS OF GAMETIC AGENTS.

Certain facts with regard to the behavior of characteristics of the parent lines in crossing are becoming increasingly evident and certain. (1) Somatic differences result from the combination of gametes of unlike potentiality in the same character manifestation, and that out of many such combinations there is interchange of the characters as far as the somatic manifestation is concerned, and, therefore, we conclude interchange in the gametic masses also, and evidence, from the derivation of pure stable lines in F_2 showing the interchanged characteristics, is from many observations in plants and animals, regarded as proof that some gametic change has taken place. This is best described and interpreted as interchange of gametic agents. (2) In all observed instances of metathesis the interchange is always between equivalent characters of the same kind, and occupying in the organism the identical location and manifestation; that is, flower characters of form or color or pattern interchange, leaf form or plant habit interchange in plants, while in animals it is coat color, pattern, activities, and so on of the same position and time of expression in the life of

the individual that cross over from one to the other of the two parental gametic systems, while the major proportion of the features of each parent retain their integrity and come out in gametogenesis, either pure or with interchanged characteristics.

This regular interchange of characteristics, the typical Mendelian reaction, is now so well established in many plants and animals from the observations of so many independent observers that there is no question but that it represents a general type of activity in the reactions of the germ-plasm in all living forms. It, moreover, is the mechanism whereby much heterogeneity is produced in any population of organisms.

The essential problem is, however, what is the cause of the interchange of the gametic factors, and why is it so regularly between equivalent productive centers of action in the germinal material? In my materials, why does *L. signaticollis* show no interchange of characteristics when crossed with *L. diversa,* and interchanges when combined with *L. undecimlineata?* In the first combination the entire gametic mass acts as a unit, and in all cases retains its integrity and identity through all operations, in all its qualities and attributes, while in the second there is interchange of larval pattern and larval body-color, each as independent units, as the regular behavior, and, under certain conditions of combinations, also show interchauge of characteristics of the antennæ, pronotal pattern, sculpture and so on.

That the observed conditions are due to any chromosome behavior, at least any that is now known, seems to me highly improbable. That the interchange is due to difference in gradient or in potentiality there is no evidence. Moreover, if it is some sort of chromosome reaction, produced by the breaking of the rods in synopsis or at some other time in gametogenesis, what is the mechanism that produces the regular random interchange and the mathematically regular classes of gametes?

It seems to me, from the many instances that have passed through my hands, that the operation is not that of any morphological action, but of some chemical transposition of materials between the two gametic masses and to be a reaction rather typical of ordinary metathesis of the type common in chemical combinations, *i. e.,* $2HCl + CaCO_2 = CaCl_2 + H_2CO_3$. In other words, the gametic materials of one parent, in certain combinations, through some purely chemical affinity, have stronger attraction for the position held by an equivalent component than for the original position, pass over to the new, displace the second, and occupy its position, while the second passes back to the position occupied by the first. Or it may be that the conditions in the united gametic masses, before their final separation, set free all of the agents of this one sign which are then free in the germinal material, and later attain to a purely chance distribution in the gametic compositions, giving essentially equal numbers of the different combinations of the two gametic bases and the transposed or one-time dissociated agents.

However it is attempted to symbolize the nature of this reaction, the fact of there being some reaction that is productive of the differences in perceptual experience with the different somas produced is beyond doubt. The most logical interpretation of the gametic portion of the phenomena, upon the basis of known chemical or physical bases, is that there has been actual passage of centers of

activities of greater or less magnitude between the parental gametic systems, and represented by integrated substance of specific composition, with specific capacity of reaction when combined with some one or more complementary agents in its new position.

The most striking fact in this metathetic reaction is that the agent always goes over to a precisely similar position, mode, and time of somatic expression, or, in other words, it goes to a basal point of attachment in the new gamete, where it finds or establishes physical relationships that hold it firmly in position and in whole or in part govern its reactions in the development of the zygote which it may help to form.

Another striking fact of these reactions is the uniform finding that the metathetic reaction is always between qualities having the same general type of manifestation, as interchange of color, of form, of pattern or symmetry, and so on, and never is there interchange of a color character with one of form, food-relations, or reproductive activity, so that we are coming to see that there are groups of these agents and of their associations, and that the Mendelian reaction is between differences in these groups, and not between different groups.

A second point that has been forced upon my attention is that it is the determining agents, the determiners, that are chiefly, if not exclusively, concerned in this metathetic reaction, while the compliment or factor in my materials never shows this type of reaction. Further, the factor is in my experience always associated with the basal species complex, firmly bound into one association, productive of the basal species complex. What these are in the composition of the gametic material no one knows, but their reactions, as I have observed them, suggest that they are possibly different colloids in the general colloidal mixture that forms the ground-substance of the living mass, and of this interpretation support is found in the fact that in my materials these factors are properties of the whole mass, and so strongly suggest a general distribution throughout the entire germinal material.

The determiners, on the other hand, are numerous, and are the agents that show not only metathesis, but also capacity for fragmentation, groups of them being dissociated in one case or lesser groups in another, and in this class of agents many interchanges, from extensive on the one hand to increasingly minute on the other, have been possible in my materials.

These agents do not behave as properties of the whole mass, but in relation to specific portions, locations, in time, in ontogeny, and in relation to specific activities, and appear to be associated with basal material points of attachment that are specific for the source from which the gametic material came. These latter seem not to be dissociated from the original mass in the usual metathetic reactions, but to maintain their association with the species complex, interchanging only the specific portions that are productive of localized determinative actions.

These experiences with my materials, and the data that has come from the investigations of the neo-Mendelians, lead directly into the problems of the architecture of the germinal material.

ARCHITECTURE OF THE GERMINAL MATERIAL.

The concept one forms of the germinal material depends to no small degree upon the nature of one's underlying philosophical viewpoint, and to a lesser extent upon the nature of one's perceptual experiences with organisms, whether descriptive and morphological, genetic, developmental, physiological, vitalistic, or mechanistic, with plants or animals.

With those who look upon living substance as something different in kind from the other integrations of the planet I have nothing in common. From what we know of this substance upon this planet I see no reason for thinking that the same order of cosmic phenomena are to be found upon any other, nor any reason for supposing that the living materials are anything other than the natural geological consequences of a zone of great and constantly changing stresses between the atmosphere and lithosphere and living objects, but the complex integrations of the materials and forces acting in ceaseless integrations of matter and changes in energy in this contact zone of intense metamorphic activity.

I must look upon organisms merely as a geological-stratum complex, ever changing in thickness and intensity of manifestation, presenting rhythmic alterations with the astronomical rhythm of the seasons, and in longer periods as earth forms change in geologic activities, and I must conceive of "life," its origin, activities, and evolution, as due to the same physical and mechanistic forces of action and result as are found in other earth phenomena, only more complicated and labile.

Through all the history of the planet there has been, as far as there is evidence, since the beginning of living things, genetic continuity of a germinal basis, relatively minute in quantity, wonderfully complex in its potentialities for producing almost endless secondary manifestations under appropriate conditions of the medium. Of this genetic continuity, first clearly put forth by Darwin, and of the existence of a genetically continuous germinal material basis so clearly presented by Weismann, there is no doubt. In this aspect, often termed self-perpetuating, this product of the planet differs from all others, but is not of itself self-perpetuating solely—only to the extent that it has presented to it in the zone of its action to requisite conditions of atmosphere and lithosphere, plus the larger astronomical relations of the planet, to produce the necessary constellation of conditions for the phenomena we call development and growth.

There is no escape from the concept that evolution and all organic activities are conditioned and governed by the relations of this germ-plasm to its environment, and that understanding of the many phenomena that we and all other organisms possess and show depend upon the discovery of the nature of the germ-plasm and of its relations to the different environmental factors that provide for its secondary or somatic modifications and for its metamorphosis or evolution.

Many concepts of the architecture of this material have been formed; no doubt many more must receive consideration before its nature is comprehended. Moreover, the problem is not the same throughout all living things, having one aspect in plants, another in animals, and different aspects within both of these

two main divisions of organisms. The remarkable specialization and localization of this material in higher animals has, no doubt, tended to restrict and codify our conception of this material, to limit it unduly; but in plants many portions of the plant tissues, serving a purely somatic rôle, may on the advent of a proper environmental reaction regulate, and from a purely somatic aspect develop, divergently into the buds and the production of gametic masses or germ-cells. In the development of the plant there had been specialization along somatic lines, perhaps development of special features of structure and function, but not complete loss of the capacity to recede from this position, to regulate, return to an initial position, and produce buds with the proper specialized germ-cells characteristic of the species. With higher animals this regulatory capacity seems to have been lost and only that portion of the zygote that enters into the formation of the gonads goes on into the next generation, while opinions differ as to what really happens in lower animals. In all, however, there is the production, by one means or another, of the necessary germinal masses or germinal materials to carry on the specific combination of qualities and attributes of the genetic lines of descent.

The development by Weismann, Nageli, De Vries, and others of the concept of a germ-plasm, idioplasm, and so on within the mass of the germ-cell, resident perhaps within the chromatin of the nucleus, is entirely an anticipation of nature and not even an interpretation.

The independent position given to this germ-plasm in Weismann's hypothesis, a microcosm within the germ-cell, the biophores being essentially living individuals, is a vitalistic conception not open to investigation, and, therefore, hardly worth consideration until there is some exact experimental demonstration of its reality. Whether the germ-plasm is a special portion of the reproductive cell or whether the whole germ-cell is germ-plasm, is at present a matter of *a priori* opinion, but it seems most naturalistic and reasonable to think of the whole germ-cell as having germinal potentialities.

The primordial germ-cells, through the division periods, seem to me to represent the real germ-plasm in its simplest condition, and are usually assumed to have the same potential composition as the zygote in which they are located, and no doubt are so, at least at the start. The organization and development of the growth period are, however, it seems to me, the initiation of ontogeny, which progresses for a certain time, but as a rule goes no further without fertilization or the influence of some external agent, in order to set up the reactions necessary to the series of ontogenetic reactions. Loeb's discoveries and those of his followers with artificial parthenogenesis show most clearly that external physical agents can provide the necessary impetus for the egg to develop without union with some other germinal mass. These results that have come from the investigations of artificial parthenogenesis show that development is not independent of external motive forces, but is entirely dependent thereon, either through normal fertilization or the action of purely physical forces, or both.

Since the discovery of the crude aspects of the mechanism of fertilization, the union of the pronuclei, we have assumed that the gametes were the same in their hereditary potentialities; but the facts of artificial parthenogenesis show clearly that at least they are not the same in their development and it is not impossible that they may also be different in their hereditary or germ-plasm potentialities.

No sperm is able to develop at all parthenogenetically; many, possibly all, ova are so able to develop. What is the significance of this from the standpoint of germ-plasm constitution?

In the terminology of hereditary reactions the two germ-cells are asserted to be equivalent and to carry essentially the same potentialities or agents. Is this really so? What evidence is there for this assumption, aside from the pairing of relatively trivial characters?

In the crossing of widely divergent types it is, as far as I know, entirely the maternal parent that finds expression in form, symmetry, rates of development, and all general properties; and only in the special determinative type of action in localized portions that the paternal type shows its influence. In my materials, where I have had, in the crossing of species, many examples of this dominance or prepotency of the female type in widely separated species, the segregations in F_2 have shown, not the presence of the paternal factor, but of the maternal factors combined with the paternal determiners, so that the segregated line, like the female parent, was the same as the original stock; but the segregated male type was quite uniformly not the same as the original type, but differed in concrete respects. In other words, experience with the materials I have used gives no indication that the male gamete carries any of the general factors of its species, but rather the determiners—that is, the female gamete carries both the essential groups of agents, its specific factors and determiners, while the male gamete carries mainly, if not exclusively, the determiner complex. An egg can develop without fertilization; a sperm can not; and may this not be the reason for the decided difference of the two as regards their capacity for development parthenogenetically? If it be assumed that the two are equivalent in constitution as regards their gametic composition, then why will eggs develop parthenogenetically and sperms will not? Further, if both are the same in composition—that is, carry all the gametic agents proper to the species—why in widely separated materials is it always the maternal parent that is dominant in form, rates of development, and symmetries, the male having influence only in the determination of special characteristics that may be common to both species? It seems to me that the question of the equivalent composition of the two gametes has been too commonly assumed to be so, following hypotheses now more or less outgrown.

Following Weismann and others, we have been prone to think of the germ-plasm as a specific single kind of material with organization into some fixed system, with the different "determiners" occupying in it a specific position, like the side-chains in some complex molecule and its symmetries compared to crystalline organization. My experiences have led to the view that it is not a single substance, but a mixture, or solution of substances which, like many other mixtures of material, shows specific symmetries and special organization characteristics. The evidence for this conception comes from experiences with the crossing of different species and the showing that there are different groups of activities, the agents in each group interchanging with similar agents in equivalent groups and with no other.

The following scheme of composition and classification of the agents in the germ-plasm seems to hold throughout my materials and presents, as far as symbolization will permit, the ideas that have been developed with regard to its

architecture. I distinguish two major types of agents: basal agents, which in all experiences are properties of the whole type and do not segregate in any of the normal reactions incident to development or in crossing, thus retaining their integrity; and definite agents, or those that are specifically concerned in the determination of the specific aspect that some quality presents. Of the basal agents there are clearly two groups, those that I have called the basal factors, few in number, and always properties of the whole, never altered or interchanged, and which may be the possible mixture of ground-substance colloids that seems to be the basis of the cell organization, and chromatic receptors, the material substratum upon which the great majority of the determining agents seem to find their distinctive seat of localization in the gamete. Of the definitive

TABLE 2.

Group.	Cytoplasmic determiner.	Basal gametic agents.		Definitive gametic agents.
		Basal factors.	Chromatic receptors.	Chromatic determiners.
1...	Vo..	PFF (phyletic form factor)	CFR'.....	Fd.
			CFR''.....	An., At., Aa., Elt.
2...	Ac...	POF (ontogenetic factor)	COR'.....	Od.
			COR'' (?)	None found.
3...	.....	PMF (metabolic factor)..	CMR'.....	Fo.
			CMR'' (?)	None found.
4...	.....	PNF (neural factor).....	CNR'.....	Ct., Tr., Hr.
			CNR''....	None found.
5...	.....	PSF (sex factor).....	CSR' (?)..	Op., Rr $^{B}_{H}$Hi.
			CSR''.....	Sx.
6...	.....	PPF (pattern factor).....	CPR'.....	J^1, J^2, J^3, J^4, Ad.
			CPR''.....	Hd., Pr., Th., Ab., Hw., Elp.
7...	.....	MCF (melanoid color factor).....	CMR'.....	Bm., Brm., Ylm.
			CMR'' (?)	None found.
8...	Mm..	LCF (liquid color factor)	CLR'.....	Wl., Yl., Ol., Rl.
			CLR'' (?).	None found.
9...	Lm..	SCF (surface color factor)	CSR'.....	Gr., Bl., Vi.
			CSR'' (?) .	None found.

agents there are two groups—a small one, always associated with the basal factors and having a distinct action as determiners of action, the cytoplasmic determiners; and the chromatic determiners, which are by far the most numerous and the agents that commonly interchange in crossing. Of these four classes I am of the opinion that the basal factors and the cytoplasmic determiners are located in the colloidal matrix of the gamete and the chromatic receptors and determiners in the nucleus.

Of the basal factors I have found nine such cases of activity, each concerned with certain general types of activity in the organisms, and with which specific determiners act to produce any given result. I have no evidence of their segregation or fragmentation, nor any evidence of their having been modified in any experimental operations to which I have subjected my materials. The combination of these nine basal factors constitutes the phyletic base—that which in the constitution has been likened to a nucleus, and in a certain sense it is a nucleus of potential activities, needing only the special definitive agent go give expression to some concrete manifestation in the organism.

TABLE 3.

Chromatic receptors, corresponding to somatic receptors.	Chromatic determiners and arrangement in groups as discovered in experiment.
1st gr. CPBS[1] ...	Fd ..... Body form index determiner.
2d gr. CPBS[2] ...	An ..... Antenna form determiner. At ..... Antenna Tolteca type determiner. Aa ..... Antenna Azteca type determiner. ElT ..... Elytral tracheal pattern determiner. ElP ..... Elytral pattern determiner (sculpture).
3d gr. CPOR[1] ...	Od.
4th gr. CPOR[2].	
5th gr. CPMR[1] ..	Fo ..... Food determiner.
6th gr. CPMR[2].	
7th gr. CPNR[1] ..	Ct ..... Chemotaxis (sex) determiner. Tr ..... Temperature reaction determiner. Hr ..... Humidity reaction determiner.
8th gr. CPNA[2].	
17th gr. CPSR[1] ...	Oh ..... Ovoposition habit determiner. Rr (B .... / H ....) Reproductive rhythm determiner {Breed. / Hibernate.} Hi ..... Hibernation determiner.
18th gr. CPSR[2] ...	Sx ..... Sex determiner.
9th gr. PPR[1]	J^1 ..... First larval pattern determiner. J^2 ..... Second larval pattern determiner. J^3 ..... Third larval pattern determiner. J^4 ..... Pupal pattern determiner. Ad ..... Adult pattern determiner.
10th gr. PPR[2]	Hd ..... Head pattern determiner. Pr ..... Pronotal pattern determiner. Th ..... Thorax pattern determiner. Ab ..... Abdominal pattern determiner. Elp ..... Elytral pattern determiner. Hw ..... Hind wing pattern determiner. Pu ..... Elytral punctation determiner. V ..... Shape determiner.

Factor.	Determiner.
MCR = Melanin color receptor.....	B. = Black determiner. Br. = Brown determiner. Ylm = Yellow melanin determiner.
LCR = Lipoid color receptor.....	Wl = White lipoid color. Yl = Yellow lipoid color. Ol = Orange lipoid color. Rl = Red lipoid color.
SCR = Surface color receptor.....	Gs = Green surface color. Bs = Blue surface color. Vs = Violet surface color.
PPS = Phyletic pattern system:	
Pl = Larval pattern factor Hd...	Hd = Pattern amboceptor.
Pr...	Pdd.
Th...	Thd.
Ab...	Abds, abdap, abdp, abdt.
Pc = Pupal pattern factor Hd...	
Pr...	
Th...	
Ab...	Abds, abdap.
Pa = Adult pattern factor Hd...	
Pr...	
Th...	
Ab...	
POS = Phyletic ontogenetic system.	
PRS = Phyletic reaction system.	
PMS = Phyletic metabolic system.	
PSS = Phyletic sex system.	

Of the somatic determiners I have found only three thus far, although further study may no doubt show more. Corresponding to each of the basal factors there are in some instances two chromatic receptors, each with its determiners. For three of these basal factors two chromatic receptors have been identified, each with one or more determining agents, and for the remaining six, one chromatic receptor with its determiners. The chromatic determiners are numerous, about 50 such major agents having been found, many of which are obviously compounded of many minor agents, or at least are capable of fragmentation and extensive modification through different processes.

It is perhaps suggestive, certainly not conclusive, that the number of basal factors, 9, corresponds with the reduced number of chromosomes in the gamete, and that for some of these there are two groups of chromatic agents, which, if it is true for all the basal factors, would give 18 such groups, which is the $2X$ number of chromosomes in the zygote, so that there is possibly some real relation between these findings; in fact, I am at present inclined to the hypothesis that there is a rather specific relation.

It seems to me that the best interpretation of the findings is that the commonly dissociated and interchanged agents are, as Morgan shows in *Drosophila,* probably located upon the chromosomes. The apparent number of basal factors in *Leptinotarsa,* 9 (with the two pairs of interacting groups of agents for three of them) suggests, but by no means proves, rather important relations that further investigation may make certain. In tables 2 and 3 I have given a list of the different agents and the symbols used in their description in the following pages:

I picture the gametic material, then, as a mixture of substances, each more or less independent and concerned in special manifestations of the whole, resulting through the numberless interactions in the correlation of form and activities, qualities, and attributes in local and general characters, producing the total array that we know. In many aspects it appears to me not unlike many another terrestrial stratum—a mixture of different materials, yet presenting generic characteristics and endless minor specific differences. Granites, compounded of certain substances, all have generic likenesses of form and structure; but with the differences in proportion of the factors of composition, with the varying action of the conditions of the medium at the time of combination, or by the press of conditions after the original production, produces granites of many kinds. May it not be profitable for a time at least to view organisms in much the same manner as a product integrated in the contact zone between the atmosphere and lithosphere as purely physical and mechanistic productions of the geological and cosmic forces incident upon the planet's surface? At the least I have tried to show the standpoint from which I must approach the problems and the concept held of the nature of the gametic material or germ-plasm, which is the basis of organic evolution and activity. I can not but feel that in the intensity of modern life and its necessary specialization we have become too intensely biologists, too little natural philosophers; that in the specialization, so characteristic of our period, there is unfortunate liability to lose sight of broader relations and points of view, and in the restriction of activities to narrower and narrower fields unintentional error and misconception unwittingly creeps in. The naturalistic point of view is true in part, it must be in all,

and there must be common concepts, underlying agents and processes, methods of production and evolution or transmutation, that should be conceived of and pondered much; otherwise, individually or collectively we are soon lost in some intellectual cul-de-sac, from which escape is difficult.

In the presentation of the data of this attempt to determine the composition of the gametic material,[1] I shall present first the results of interspecific crosses, first those of the simplest reactions, later those of more complicated characters; second, the findings in intraspecific crosses, especially the experiments that have shown the nature of the chromatic determiners and their various capacities for fragmentation; and at the end attempt a further analysis of the general aspects of the findings and their bearing upon the problems of germ-plasm constitution and architecture.

[1] The complete account of the gametic analyses made with these materials is in process of preparation, to be presented in a separate report.

CHAPTER IV.

REACTIONS AND PRODUCTS IN INTERSPECIFIC CROSSES.

I have had to deal with many interspecific crosses, and these I have uniformly treated as follows: Species freshly introduced into the laboratory were first acclimatized to the conditions of the breeding-quarters, which were the average conditions of the breeding-season of their native habitat. It was found that 3 to 6 generations will suffice to fully adjust any wild stock to the conditions of the breeding and experimental rooms. In this way I eliminate in crosses between species many of the disturbing effects of *conditions* in the material, "adaptation" to one especial complex, and thus make the crosses the product of the constitution of the organisms as completely as is possible at present. I was forced to do this, because I found that species direct from diverse habitats in nature were giving most irregular results that were difficult of repetition and analysis.

It may be objected that this treatment is abnormal, and that it is productive of change in the species; but none has been found, and only those species are used for these interspecific crosses that are able to completely adjust themselves to one environmental complex. The mere fact that they are not able to do so is all the indication needed to exclude them from crossing experiments where effects of environment are to be eliminated. It has been my experience that species so brought into the same set of conditions do not change; do not lose any capacities or characteristics, are not weakened, and when returned to the original specific native environmental complex are able to take up at once the original routine without effort or waste of individuals or energy.

METHODS OF CULTURE.

The most essential factor in the successful breeding of animals of the kinds that I have used—phytophagous Coleoptera, Lepidoptera, and Hemiptera—is the constant presence of a plentiful supply of perfectly fresh and normal food, under conditions such that the normal transpiration of both plant and animal can go on, *and with adequate air-movement to remove from the vicinity of the organisms the air contaminated by the products of respiration and transpiration.* It has been my experience that unless this is attained in a complete and constant manner trouble will inevitably follow in the cultures—disease, weakened vitality, with the probable loss of the culture and termination of the work.

In all experiments it has been my first care to have the organism, regardless of the kind upon the specific *living plant,* which is the normal food for the species in the location from which the material originally came. The plant must not only *be alive,* but in the usual *actively growing condition,* as in nature, so that the organisms may have an adequate, normal, and hygienic food supply.

FIG. 1.—Photograph of breeding-cages used for pedigree cultures, showing arrangement of the cage, the large earthen pot, and the plant used as food, the use and relations of which are described in the text.

Cut food wilts in a short time and with especial rapidity under high temperatures or under arid conditions, and attempts to keep the food fresh by placing it in water end in failure or in indifferent results at the best. When a healthy growing plant is used as the food, the animals always breed and lay their eggs exactly as in nature, and in my experiments over 90 per cent of the fertilized eggs laid (by actual count) hatch, and the freshly emerged, delicate larvæ can at once begin feeding in a perfectly normal manner without in any way being molested or injured.

The most satisfactory type of cages thus far devised for this work consists of a light, strong drum of fine wire-netting (pearl wire, No. 22 mesh), of which the sides and top are of wire and the bottom of galvanized iron, with a sleeve that projects down into the earthen pot that serves as the base as shown in figure 1. The pots that I have used are 12 inches in diameter and hold a considerable volume of earth, in the center of which is placed the food-plant in a smaller pot. The breeding-cages are uniformly 18 inches in diameter and are either 18 or 24 inches in height. A cage of this character is used for each mated pair and for their eggs and young progeny. With this arrangement the breeding pair has normal, healthy, and fresh food, proper conditions for egg-laying, and uncrowded space about them. They have for all practical purposes natural conditions for their breeding activities.

I allow one pair to breed in such a cage, and when the progeny are half grown they are put into sorting-cages in which the same construction is employed, excepting that they are only 12 inches in diameter (fig. 1*c*); though if the progeny are especially abundant or valuable the parents are removed to a fresh cage to breed further and the first 100 or 200 are allowed to complete their ontogeny in the original cage. As soon as the food-plant is defoliated a new one is easily put in its place.

Entrance to the cages is had by means of a sliding door in the side, large enough to permit the passage of an 8-inch pot and food-plant. This type of breeding-cage has the advantage that it is self-sustaining, and needs but little daily attention aside from the routine spraying of the plant and the adding of a regular amount of water to the soil in the pot. The mechanical labor of the project is thus reduced to a minimum, with constant efficiency of operation. With these arrangements I have been remarkably free from epidemics of disease. The only epidemic that I have had to combat was not a very serious one. It was introduced during my absence and was not recognized by the assistant in charge, because of his never having encountered anything of the kind before.

The larval stages are thus passed under optimum and normal conditions and they pupate in the soil of the same cages in which they pass the latter portion of the larval period. As a rule it has been found advantageous to keep the later stages in the sorting-cages, which occupy less room and leave a larger number of breeding-cages free for the mated pairs. After pupation the cage is watched with care to keep the soil at the optimum condition as regards moisture, and also for the adults to emerge. These are isolated at once, the males from the females, in separate sterile cages, and given living food as in the breeding-cages. This separation is made two or three times daily during the period of emergence, within 1 to 4 hours after emergence, and before the post-pupal development has been completed. The emerged adults are without exception allowed to remain in these cages, with the sexes separated from one another, from 5 days

to 4 or 5 weeks before they are mated for the next generation. This period of feeding and inhibition from breeding gives the stock an opportunity to become fully nourished before they begin the breeding activities, and also serves to eliminate the weaker members of the population, which will uniformly die within a few days of emergence. This procedure insures an almost certain and uniform breeding from strong and well-nourished individuals, with the result that inbreeding can be practiced to a far larger extent than if the more usual custom were followed of mating the individuals as soon as they were ready to breed. Another advantage of this method with the sexes isolated and upon normal fresh growing food is that the stock can often be held for months without serious consequence ensuing. It is thus possible to breed part of the population, hold the remainder in reserve for 3, 6, or even 10 months, and thus be able to check the first matings or to make other combinations not made at the start. This is of immense advantage in the working out of the results of hybridization.

All of my pedigree work with tropical species has been done in large glass houses, with high walls, giving a large volume of air, in which the temperature was controlled automatically, and arranged to correspond with the average night minimum of the growing season in the natural habitat of the species undergoing investigation at the time. The maximum at mid-day is of less importance and in the summer months was variable, in the sun often from 29° C. to 35° C. and in the shade from 26° C. to 30° C., which is comparable with the natural conditions found in nature in the tropical regions from which the material came. The humidity was controlled either by the amount of water available for evaporation and by the water of transpiration set free in the air by the plants in the room, whose evaporating surface amounted on the average to about 25,000 square feet of leaf-surface, or by special humidity control. The actual water-content of the air remained constant or nearly so, but with the range in the daily temperature there was of course a change in the relative humidity of the air of the room and, therefore, of the evaporation from the surfaces of the organisms contained in the cages.

The food-supply is an important and vital portion of the project and must be met by growing the food appropriate for the species and maintaining a constant supply of it, so that at any time matings can be made as the animals are ready therefor, or when any portion of an experimental series needs to be analyzed. These are best grown as potted plants, after the usual routine of florists, and must be kept free from infestation by pests or the presence of eggs, larvæ, or the adults of any of the different species that are under investigation. I have grown my food-supply in an ordinary glass house that was separated from the room where the animals were kept. The food was kept sterile by frequent fumigations with HCN gas and by sprayings with solutions of nicotine. This has given complete protection against the possible introduction of any stray individuals into the cultures.

Many of the food plants that I have used will last for a long time, a year or more, and will give several crops of foliage in the course of their life, thus materially reducing the number of plants that must be grown in the course of a year to maintain the project. My experiments at Chicago have required in the neighborhood of 10,000 plants per year, and a constant supply of from 2000 to 3000 plants must constantly be maintained.

Frequent sterilization of the pots, soil, and cages by live steam under a pressure of 10 to 20 pounds for periods of 4 to 10 hours for each sterilization has materially contributed to freedom from disease, parasites, and kindred troubles in both the animals and in their food plants. For this purpose steel drums have been used. These are about 3 feet in diameter and 4 to 6 feet long, and are connected to the steam mains that heat the quarters used for the experiments. Live steam enters at a pressure of 90 pounds and with a temperature that ranges from 163° to 170° C., and then dropping to a temperature of 108° to 113° C. for the temperature of the sterilizer.

With properly sterilized cages, good food, and healthy stock, the chances of numerous and vigorous progeny are as good as in nature, and I have often suspected that in my cultures the progeny really surpass in number and in vigor their less favored brethren in nature. Once a culture is set up, the maintenance thereof is not difficult nor time-consuming. Only a moment is required daily in which to thoroughly spray the cage and the food plant with the hose and to add to the soil a fairly regular amount of water in order to maintain optimum conditions of growth. This in general is the method of handling pedigree cultures that I have used, and it has uniformly given satisfactory results.

I have been especially interested in the relation of the Mendelian reaction to the problems of species and groups in nature, and the question whether in the crossing of species a special order of reactions is shown. All of the older workers seem to be of this opinion. De Vries clearly thinks of the Mendelian reaction as present only in those forms standing in the relation of species and variety, while elementary species or specific groups present a different type of reactions. The same conclusion is expressed by many observers as the result of diverse experiences.

The materials that I have used all come direct from nature, have never been under domestication, nor in any way subjected to the operations of man. They are natural species, and some of them have been known for more than a half century. With this material I have made sundry studies to determine whether in the crossing of species the reactions and laws of action were different from those found in the lesser groups in intraspecific crosses. I could discover no *a priori* reason why this should be so; but the common expression of opinion that it was so made the test necessary for the investigations that were in progress.

Species differ in diverse, concrete qualities; some that are not easily expressed in terms of "character," as for example, the "sense of difference," one soon learns to recognize, even though one is not able to state it in accurate terms. There is the total type aspect, with which are associated certain more or less easily described characteristics. I have been so fortunate as to find in the material that I have used species that could live with success under the complexes provided in my laboratory, and that showed in these interspecific crosses an array of characters such that it was possible to get a picture of the operations in the crossing of species. The general outcome of the entire series is that in this set of organisms there are no differences in the crosses of species that differentiate the reactions between species from intraspecific crosses. In all respects the principles of factorial composition and reaction are fully confirmed, and many instances hopelessly confusing at first, and contrary to the findings in intraspecific crosses, have been solved, in no instance with any discord in principle.

It is not of any interest to present or even record the "dominant" and "recessive" "characters" and the arrangement of them in schemes of their "epistatic" or "hypostatic" relations, but my object has been the development of an understanding of the structure of the gametic system, the nature of the zygotic reaction complex (soma), and the basis of the operation of the different agents involved and their relation to internal and external conditions, as an aid in the further investigation of development, transmutation, and evolution in these organisms when used as test objects. In the investigation, characters that are "morphological," those that are "physiological," have been investigated, although I can not decide where one "class" ends and the other begins; both in their productive agents and in operation are the same in all respects, save in the resultant manifestation in the living mass.

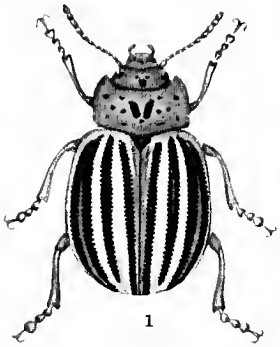
LEPTINOTARSA SIGNATICOLLIS × LEPTINOTARSA DIVERSA.

Crossing of these two species has given the simplest condition that I have found in the crossing of natural species. As shown in Chapter II, the two species are similar in many of their characters, especially in the juvenile stages; the most conspicuous visible difference is the presence in *L. signaticollis* of the elytral stripes and their absence in *L. diversa*. To this must be added the specific difference in general appearance, shape, and size, which is best determined and expressed in biometric determinations of the form index. Nowhere, as far as is known, do the two species live in the same habitat or in the same environmental complex, *L. signaticollis* living in the Rio Balsas Valley on its eastern side, *L. diversa* along the edge of the Mexican Plateau on both the east and west sides. My materials for experiment with *L. signaticollis* Stål all came from the Rancho Basoco location near Cuernavaca, State of Morelos, *L. diversa* n. sp. from the Cerro Borrega colony at Orizaba, State of Vera Cruz, the two separated by the high, dry, plateau of southern Mexico, where life is impossible to both species.

Stocks obtained from these locations, taken to the laboratory, were reared under the same conditions for 4 to 6 generations, and then utilized in the experiments first described. No effect resulted from the change from nature to the laboratory conditions except altered rates of growth, both living in perfect health, giving vigorous offspring in large numbers, in long, continued series, with the same growth-rates after 4 to 6 generations.

THE NORMAL REACTION IN CROSSING.

When these two species are crossed under the conditions of the breeding-quarters, between stocks that have become adjusted to like conditions with the same rates of development, a most regular and stereotyped series of results are obtained, irrespective of the direction of the cross, age of the parents, and strength or vigor of the mated pair. Crosses of old and young are completely without effect upon the progeny, as far as any discoverable effect upon either characters or reactions is concerned, nor is there any indication that the gametes show changes in constitution or reaction with age, or changes corresponding to or correlated with ontogenetic changes in the parent of any sort. I made especial effort to check this aspect of the crossing, that I might eliminate the "interpreting" of irregularities that might be found as due to ontogenetic or



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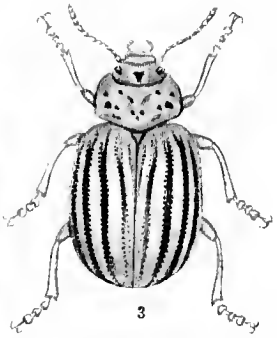

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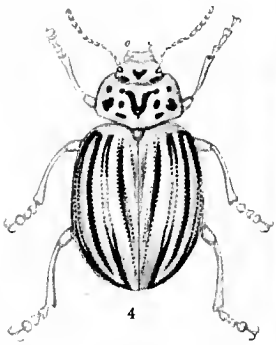

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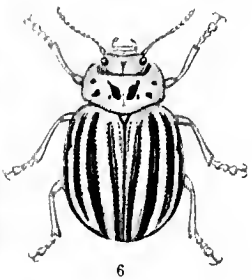

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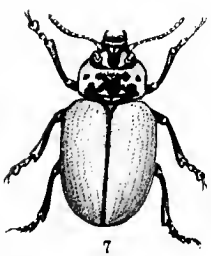

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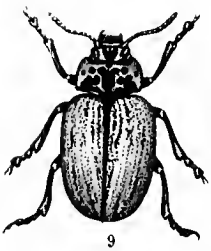

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K. TODA DEL. JULIUS BIEN, N. Y.

1. *L. juncta.* Showing elytral punctation.
2. *L. decemlineata.*
3. *L. texana.* Showing elytral punctation.
4. *L. defecta.* Brownsville, Texas.
5. F_1 heterozygote of *L. signaticollis* × *L. diversa.*
6. *L. tumamoca.*
7. *Signaticollis* type extracted from marked heterozygous race.
8. *L. decemlineata.*
9. Modified *signaticollis* type from marked heterozygous race.

orthogenetic modifications in the constitution of the gametes during the life of the individual. In these forms there is no rhythm in the constitution of gametes produced, and the germ-cells are identical in constitution and array at all portions of the reproductive period, as far as I can determine.

When crosses are made between the two species, in either direction, with pedigreed, guarded parents, under conditions common to the two stocks, the result in F_1 is the uniform production of an intermediate type that is truly intermediate in all respects, as shown in figure 7, plate 8.[1] When these are inbred they uniformly produce F_2 fraternities that in all instances show complete segregation in the proportions of: *diversa* 1: mid-type 2: *signaticollis* 1, in remarkably close approximations to the expected arrays, not infrequently in perfect proportions of 1:2:1. Both of the extracted forms are distinctly cut off from the heterozygous type and are the entire type extracted, and are not the separation of the elytral or other characters alone. The extracted types when crossed back upon the parent stock show no indication of difference in composition or in any other respect as the result of its passing through the cross. *The total type has remained as a unit* in the gametogenesis of the F_1 heterozygotes, no interchange of characters taking place between the two systems, although there are many that might have done so, so that the F_2 array shows the two parent forms as unaltered extracted F_2 types, and the mid-type, which is heterozygous.

The results obtained from the crossing of these two species under neutral conditions, and also a complete testing of the crossing in all combinations, everywhere and at all points in the series, show that the two types come out of the cross with perfect regularity, unchanged, showing most constant and regular behavior and retention of the integrity of the gametic systems throughout. Each acts as a "single character" in the cross, and the entire series presents the aspect in operation, as in results, of a typical monohybrid reaction, in which the contrasting elements are the two gametic systems present, one from each species. In table 4 are given some of the results of the breeding of these two species in this cross, and the working-out of the relations in F_2 and F_3, showing the uniformity in close approximation of the arrays to the theoretical proportions of each type in the population. I have bred the heterozygous types through to F_{12} without any change in the array presented or deformity in the proportions.

The extracted types in F_2 and following generations were always true to type without exception. When crosses were made between the F_1 heterozygotes and either of the parent species—and the same is true of a heterozygote at any point of any origin in the series—they give in F_1 a 1:1 ratio of heterozygotes and extractives of the same kind as the parent stock used. These, when bred out in F_2, showed that the heterozygotes always gave a typical and close approximation to the 1:2:1 F_2 array, while the extracted type came true in all. Identical results came from the crossing of the extracted types and any of the heterozygous individuals. Crosses between any of the extracted types and the natural species gave, if of like types, uniform lines; if of unlike types, the characteristic F_1 heterozygotes and the F_2 array seen in other crosses; and likewise crosses between

[1] Compare plate 8, figure 7 with parent species, *L. signaticollis*, plate 8, figure 9; plate 1, figure 9.

TABLE 4.

P_0	F_1			F_2 observed			F_2 expected			F_2 types of matings						F_3 observed			F_3 expected		
										DD×DD Div.×Div.	DR×DR Mid.×Mid.	RR×RR Sig.×Sig.	DD×DR Div.×Mid.	DR×RR Mid.×Sig.	DD×RR Div.×Sig.						
	No	Appear	Mated	Div. type	Mid. type	Sig. type	Div. type	Mid. type	Sig. type.							Div.	Mid.	Sig.	Div.	Mid.	Sig.
Mated L. signaticollis Biotype 7 × L. diversa Biotype 7			A	108	219	109	109	218	109 ⊗	A, B, C, D, E	All F_2 ext. div. types					96			100%		
			B	⊗												14			100%		
			C	39	79	39	39.25	78.50	39.25 ⊗							⊗			100%		
			D	67	135	65	66.75	133.50	66.75							43			100%		
																171			100%		
			A	⊗						F, G, H, I	F_2 mid. types mated					9	22	11	10.5	21	10.5
			B	4	4	4	3	6	3 ⊗							86	161	92	84.75	169.50	84.75
			C	8	6	3	4.25	8.5	4.25 ⊗							41	80	40	40.25	80.5	40.25
Div. ♀ × ♂ sig. H.851 A.	199	Mid.	D	⊗												7	10	6	5.75	11.50	5.75
			E	2	6	2	2.5	5	2.5 ⊗									12			100%
Div. ♀ × ♂ sig. H.853 E.	95	Mid.	F	7	27	7	7.75	15.50	7.75	J, K, L, M, N	All F_2 ext. sig. types mated							⊗			
																		39			100%
																		77			100%
																		18			100%
			A	⊗						O, P, Q, R, S	F_2 ext. div. ♂ × ♀ mid. F_2					21	22		21.5	21.5	
			B	⊗												46	49		47.5	47.5	
			C	⊗												17	20		18.5	18.5	
			D	⊗												55	50		52.5	52.5	
Div. ♂ × ♀ sig. H.451 A.	93	Mid.	E	13	18	10	10.25	20.50	10.25							92	90		91	91	
			F	11	10	5	6.5	13	6.5	T, U, V, W	F_2 ext. mid. ♀ × ♂ sig. F_2 ex.						40	41		40.5	40.5
			G	10	30	8	12	24	12								11	13		12	12
			H	⊗													55	53		54	54
																	77	74		75.5	75.5
Div. ♂ × ♀ sig. H.453.	296	Mid.	A	96	201	93	97.5	195	97.5	X, Y, Z	F_2 ext. sig. ♂ × ♀ div. ex. and F_2						23			100%	
			B	71	145	77	73.25	147.50	73.25								106			100%	
			C	144	287	143	143.5	287	143.5								44			100%	
			D	210	419	211	210	420	210												
			E	19	40	20	19.75	39.50	19.75												
			F	231	464	231	231.5	463	231.5												
			G	77	146	73	74	148	74												
			H	48	96	48	48	96	48												
			K	83	160	77	80	160	80												
			L	11	25	12	12	24	12												
Total F_1 in four matings	683		Totals	1259	2517	1237	1250.75	2501.5	1250.75												

⊗ Died or no progeny.

the extracted types gave, no matter what the point of origin nor what the history, the same uniform results obtained in the crossing of the original stocks.

That there might be no personal equation involved in the series presented after the initial combinations were made, the censuses of the fraternities were made by a technical assistant, who was not instructed what to look for, but to separate the fraternities into the different groups that he could recognize, and to isolate any that were questionable as to where they belonged. After this was done I took the materials and checked the counts and determinations of the array; any that we did not agree upon where used in breeding to test their character. Likewise, matings of the different portions of the fraternities were random, the separated classes of a fraternity being put into glass jars and the pairs drawn at random for the matings. The results presented are as free from the errors of personal equation as is possible.

From the inspection of the F_1 and F_2 fraternities that came from the crossing of these two species, as well as the extracted types in F_2 and in later generations, the impression was forced upon the observer that the total type had remained as a unit, segregating out in the gametogenesis of the heterozygous forms. There

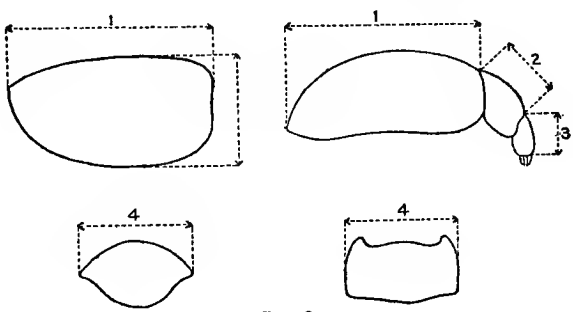


FIG. 2.

was no evidence of the interchange of any characters between the species discoverable by inspection. To test this point, several of the fraternities were investigated, as also the parent species of the stocks used in experiment and from nature.

For this purpose I studied the form-index and its behavior in the materials, in that it is a good indicator of the persistence and integrity of the gametic type. With each type are associated many minor characteristics, and these, together with the form differences, make for a distinct difference between the two species in general aspect as well as in special characteristics.

This form-index is determined in these materials from measurements of certain parts, not liable to change in drying in preserved specimens or by changes incidental to reproduction, or other ontogenetic processes. In figure 2 I have shown the measurements that were made of the adults, and the index as used was determined by the summation of the values 1, 2, and 3, divided by the value 4. These readings were made with special vernier calipers reading to tenths of a millimeter. Experience has shown that this index is the most reliable and least variable, as well as the least liable to distortion by ontogenetic or post-mortem changes in the specimens examined.

The form-index of the two species was first determined from a series of random samples, and the results are shown graphically in figure 3, showing the low range of variability of the value, as well as the distinct grouping of the two species about separate modes. The overlapping of the two polygons does not seem to have more than the significance of ordinary fluctuating variations of integral variates, and although the attempt has been made to breed pure lines

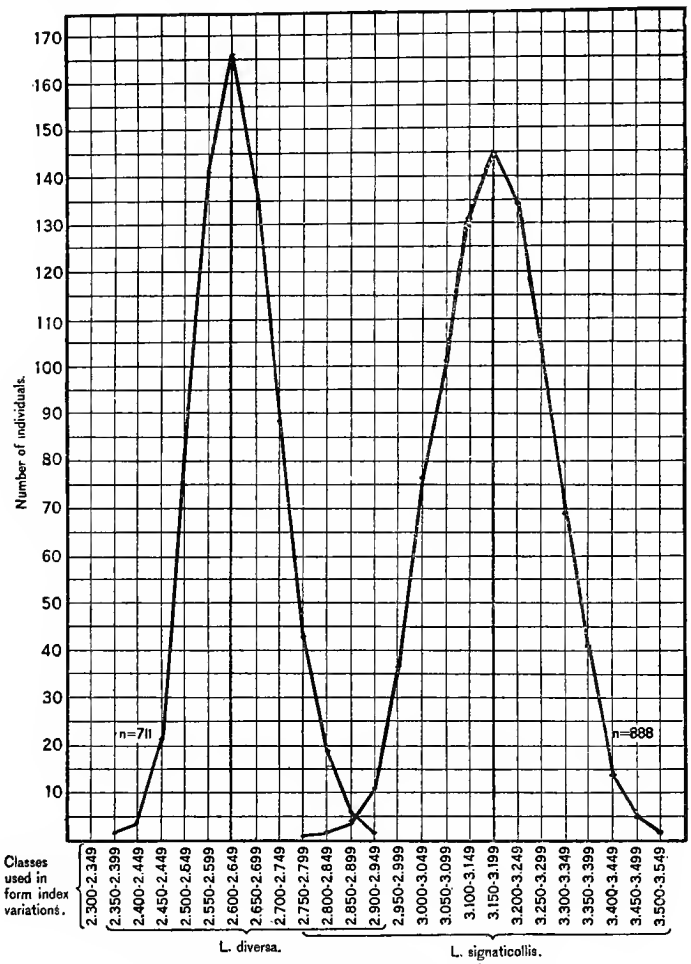


FIG. 3.

from the extremes of these polygons, no results have come therefrom, so that as far as experience goes the value is a single one, with the fluctuation common in graduated varieties.

In that this first determination was made upon materials from many localities and in different years, confessedly heterogeneous materials as regards genetic relationships, a second study was made upon materials from the two locations most used as the source of materials for this work.

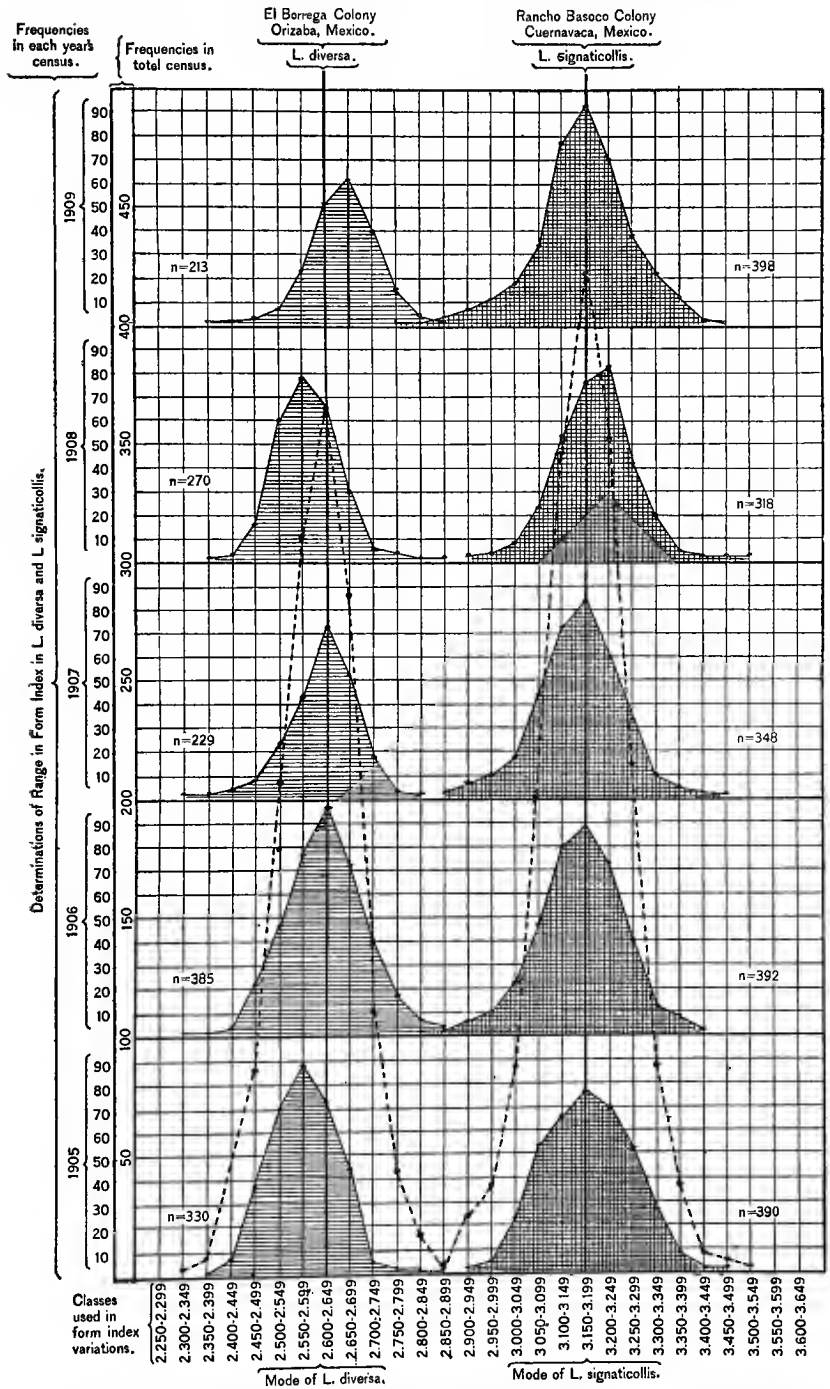


FIG. 4.—Showing results obtained in the determination of the variability in form-index in the pure parent stocks of *L. diversa* and *L. signaticollis* and by the similiar treatment of the peculiar pure-breeding extracted race that was superficially *L. signaticollis*, but which showed itself to be made up of three modal groups as far as form-index was concerned. (See text for explanation.)

In figure 4 I have shown in condensed schematic form the results of this study of *L. diversa* at the source of material at Orizaba and of *L. signaticollis* at Cuernavaca, through the years 1904 to 1909. This study of the form-index in each of the two sources of materials shows clearly that the condition is not only stable for the species in the location, but also that, taking the location as a

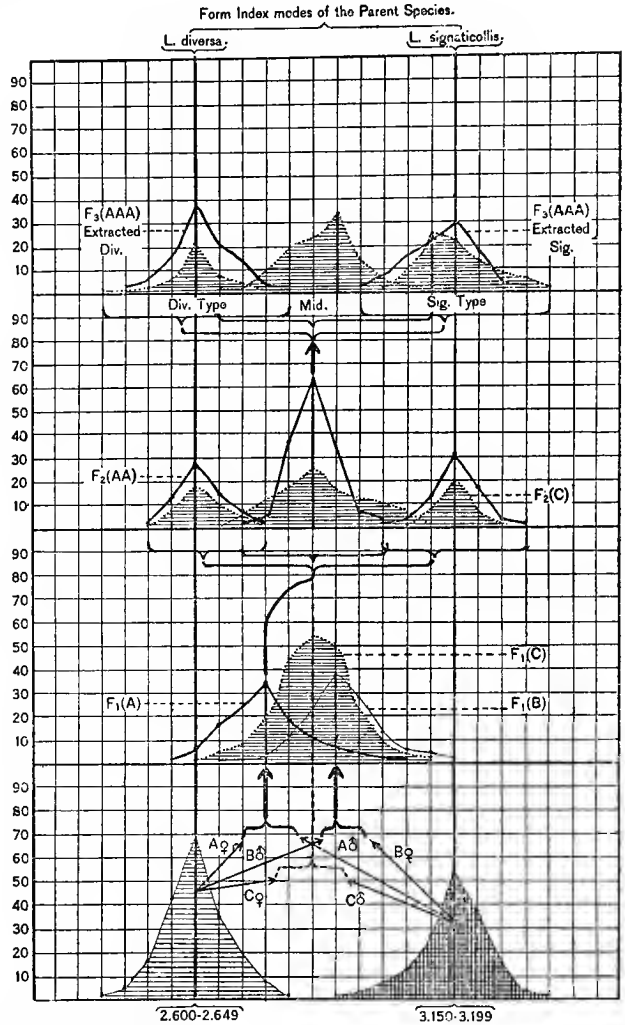


FIG. 5.—Diagram of apparatus for control of relative humidity in the breeding room. The figure shows the complete apparatus. (For explanation of its parts and working see description in the text.)

whole through the period of observation, it has the same index as determined from the random samples shown in figure 3. Some divergence of the polygons of distribution in each of the species is shown for the species mode in different years, but no divergence of this sort has been found to have any permanency,

either in nature or in experiment, and may be, therefore, regarded as the results of aberrations induced by incident conditions during ontogeny or as the result of small numbers observed, the modal divergence in different years being purely a chance one.

The inheritance of the form-index and its behavior in crossing is shown graphically in figure 5, where the products of three crosses are shown between these two species, as well as the fraternities from which the parents of each cross came. Modal individuals were used for the matings in each of the three shown. From figure 5 and table 5 it is seen that the F_1 heterozygotes are in modal position between the modes of the parent species, are intermediate in this respect, although the exact position of the mode upon the scale of values differs in different fraternities, and the range of the polygon in all instances overlaps that of the parents to a varying extent.

TABLE 5.

No.	Mode of *L. diversa*.							Mode of *L. signaticollis*.						Remarks.
	2,600–2,649	2,650–2,699	2,700–2,749	2,750–2,799	2,800–2,849	2,850–2,899	2,900–2,949	2,950–2,999	3,000–3,049	3,050–3,099	3,100–3,149	3,150–3,199	3,200–3,249	
1....	1	1	3	10	16	28	46	31	17	6	2	...	...	*signaticollis* ♀ × ♂ *diversa*.
2....	...	2	1	4	9	5	3	3	1	...	1	...	...	Do.
3....	...	1	4	10	14	40	61	42	12	5	2	1	...	Do.
4....	1	5	7	11	28	35	26	13	9	3	1	2	...	Do.
5....	...	1	1	1	4	16	31	14	5	1	1	...	...	Do.
6....	1	...	3	8	17	40	71	42	20	11	5	1	...	*signaticollis* ♂ × ♀ *diversa*.
7....	...	1	1	5	5	12	19	11	4	1	2	1	...	Do.
8....	...	1	1	2	2	7	3	2	1	2	...	...	...	Do.
9....	1	2	1	4	3	7	15	8	5	1	2	1	1	Do.
10....	...	...	2	3	13	30	46	31	15	4	1	...	...	Do.
	4	14	24	58	105	220	321	197	89	34	17	6	1	Total, 1,090.

Matings of the heterozygotes show uniformly in F_2 that the visibly segregated products are also segregated with respect to the form-index, and that the form-index of *L. diversa* is in all instances associated solely with the more easily visible characters of that species, and that none of the individuals with this index carries *signaticollis* characteristics; the same is also true of the extracted *signaticollis* type, in that it is entirely associated with *signaticollis* characters. The mid-types, or heterozygotes, in their mode and distribution, are always in an intermediate position, and individuals from this polygon always give in F_3 the same behavior as is found in the progeny from the F_1 heterozygotes. In other words, there is complete segregation of the form-index as well as of all of the characteristics that accompany it in the parent species, and no evidence has thus far been discovered in these normal crosses of the interchange of any characteristics between the two gametic systems as the result of passing through this normal crossing reaction.

The point of special interest is the behavior of the *gametic system species as a unit in crossing under the conditions of the experiment, and the non-dissociation of it in any portion of the series.* There are present the two basic species

gametic complexes, between which characters might be shifted, producing in the end a considerable array of types in F_2 and F_3 differing in small but none the less distinctive and permanent aspects. No such metathesis is observed in this series, and I believe that this type of reaction represents the simplest that can exist in the crossing of species.

It may be objected that the materials are not species because they cross and give fertile F_1 and F_2 progeny, and the fact that they cross at all would indicate to many a lack of specific distinctness. Whether the above objection is tenable or not need not be discussed, as it is simply a matter of anticipatory definition. When the species come into the laboratory from nature they are less fertile and often cross with difficulty, producing quite different results, as will be shown presently. They are reared and crossed under the conditions of experiment given, that is, under the mean average of each chief environmental factor of their breeding-period in nature, so that the conditions of experiment may not introduce any incidental agents that might change them; only the rhythm of native habitats is changed, and instead of the pressure of the brief season in the habitat of *L. signaticollis,* both are given continuous mean breeding-season conditions, to which the species adjust themselves without discoverable change except in the rates of reaction in development. As the two become attuned to the same conditions in the medium, their interfertility increases until in the end the fertility is nearly as high in interspecific crosses as it is in pure-line matings. When this state is reached the reactions shown in the crossing of these two species are uniform and represent the true basic reaction of the two gametic systems when combined in crossing under neutral conditions of the medium, and it is the reaction of the systems in simplest terms under conditions that permit of the manifestation being entirely the product of the composition and structure of the interacting masses.

Crosses of the kind indicated between these two species have been repeated yearly since 1907 as part of the annual routine to test this point and also the condition of the stocks, with no indication of any differences whatsoever. These yearly tests are random matings, with random testing into F_2, and sometimes into F_3 or F_4, depending upon the space and help available.

MODIFICATIONS OF THE NORMAL REACTION.

If the crossing of these two species presented only the behavior shown, it would be of itself of interest because of its simplicity and the integrity of the gametic systems shown, but this is not the reaction shown when the materials are fresh from nature and not adjusted in their reactions to the same set of external conditions. I found in 1904 that these two species, when fresh from nature, in guarded parentages, gave in F_1 a behavior that suggested that one of the parents was heterozygous, the other homozygous with respect to the elytral striping, with the result that in F_1 two classes of about equal numbers appeared. Thus when the cross was made between *L. signaticollis* female and male *L. diversa,* the uniform result in F_1 was about 50 per cent of *mid-types* and 50 per cent of the female *signaticollis types,* and these when inbred in each class gave in F_2 and in subsequent generations the extracted female *signaticollis type,* "breeding true" and the *mid-type* splitting in F_2 into *signaticollis, mid-,* and *diversa* types in the usual ratios. This result I have shown in plate 9. It is in every way typical and suggestive of the crossing of a DD × DR or DR × RR.

This condition was uniformly present in the crosses of the first and second generations in the laboratory, but in the following generations this behavior changed progressively with decreasing percentages present of the female *signaticollis* type, until at the fifth or sixth generation none was present, nor did they appear again in the crossing of the two species as long as the stocks were maintained under like conditions. There is shown in this series of observations a condition that might be of much importance that would produce unlike results in different locations in nature from the crossing of the two species, permanently altering the history of some individuals. I undertook, therefore, a careful examination of the stocks and attempted to experimentally produce the modification in behavior that was found.

Careful examination by measurements, by crossing back upon the fresh stocks from nature and upon older strains that had been longer in the laboratory, failed to show any heterozygous condition present in the gametic constitution of the stock species that could be detected.

TABLE 6.

Stock No. 419 (*L. signaticollis*).				Stock No. 817 (*L. diversa*).			
Nature.		Chicago.		Chicago.		Nature.	
Period.	Days.	Period.	Days.	Period.	Days.	Period.	Days.
June 2 to July 17.	45	April 18 to June 10.	53	Nov. 10 to Jan. 6.	57	May 4 to July 5.	63
May 26 to July 5.	39	Nov. 2 to Jan. 4.	63	Sept. 30 to Jan. 1.	62	June 8 to July 8.	61
Aug. 2 to Sept. 6.	34	Sept. 20 to Dec. 15.	65	Nov. 2 to Jan. 6.	65	July 4 to Sept. 4.	62
June 30 to July 10.	40	Nov. 2 to Jan. 6.	65	Mar. 11 to May 11.	60	April 29 to June 30.	62
July 25 to Sept. 2.	39	Mar. 11 to May 11.	61	April 6 to June 5.	60	April 24 to June 26.	60
June 4 to July 10.	36	April 13 to June 4.	62	Mar. 11 to May 11.	61	May 14 to July 20.	68
June 7 to July 17.	40	April 13 to June 8.	66	April 8 to Jan. 5.	58	June 4 to July 12.	69
Aug. 5 to Sept. 10.	36	Oct. 7 to Dec. 6.	60	April 1 to June 1.	61	May 27 to July 31.	65
Aug. 1 to Sept. 4.	35	Sept. 1 to Dec. 2.	61	May 11 to June 9.	69	June 1 to Aug. 6.	65
May 24 to July 31.	38	Nov. 2 to Jan. 4.	63	April 5 to June 1.	57	May 31 to Aug. 2.	63
Average.....	38.2	Average.....	60.9	Average.....	60.2	Average.....	64.1
Records made at Cuernavaca of stock from Rancho Basoco Colony.		Records made, taken from records at random, between 1907 and 1912 and are of the behavior after four or more generations in the conditions of the breeding quarters used.				Records made at Orizaba of stock from Cerro Borrego Colony.	

In nature the average length of ontogeny in *L. signaticollis* is about 40 days and in *L. diversa* it is about 60 days. In the records of my cultures I found that the stocks fresh from the natural habitat retained the same rates under the conditions of the quarters at Chicago in the first generation there, but that they progressively changed in this respect, so that at the sixth generation at the latest, often earlier, they had come to have the same rate of development and the same length of ontogenetic period from egg to the adult. In table 6 are given data taken at random from the records showing the rates of development at Cuernavaca, State of Morelos, Mexico, for *L. signaticollis* Stål, at Orizaba, Vera Cruz, for *L. diversa* n. sp., and the rates in two stocks at Chicago that were obtained at these locations after the period of probation had passed, when the crossing of the two gave the monohybrid results described. At this time both had the rate of 60 days each, a rate retained indefinitely under laboratory conditions as far as known.

In the natural habitat at Cuernavaca, the growing-season, from mid-June to mid-September, 90 days, allowed only the requisite time in the year for the species to complete its two annual generations and prepare for the oncoming of the dry season in September. In the habitat of *L. diversa* at Orizaba the growing-season was longer, from early June to the late autumn or even into January, giving the species ample time to undergo its development before the onset of the dry season. In the laboratory the average growing-season conditions of the habitats were maintained throughout, so that there was no pressure to hasten ontogeny in the case of either. The uniform result under laboratory conditions is to slow down the rate of development of *L. signaticollis* and to slightly hasten that of *L. diversa,* both coming to have the same rate of ontogenetic progression.

It would seem, therefore, that this condition in the stocks on introduction from nature might be productive of the difference found in the crossing on introduction and after establishment. If this were true, it then ought to be possible to produce in experiment, from races that were giving the monohybrid behavior, the conditions shown in plate 9. It ought also to be possible to discover something of the mechanism of the production of the difference, especially in the production in F_1 of the "pure-breeding" types.

To eliminate the possibility that the condition was the product of a local difference, in the spring of 1907 I brought to the laboratory from Atlixco, State of Puebla, Mexico, fresh materials of *L. signaticollis* Stål and of *L. diversa* n. sp. from Guadalajara, State of Jalisco. These I obtained in late June in the larval condition, and they were taken to the laboratory as pupæ and emerged, the sexes being immediately separated. A generation fresh from the field, representing random materials, but in which the virginity of the mates was absolutely certain, was available and at once crossed, with the result that the different crosses of the *signaticollis* × *diversa* gave uniformly the same result as had been obtained many times then and since from stocks from the two other locations. These I continued breeding and crossing at random through the seasons of 1907, 1908, 1909, and 1910, with the results shown in table 8, showing in all respects the same behavior that was found in all of the other stocks. This result made it certain that the condition was a general one and not due to special local conditions.

In the years 1908, 1909, and 1910 the same test was made with new stocks from the Rancho Basoco and Cerro Borrega locations, with the same results, showing close agreement in the progressive change in the rate of ontogeny, and with this a similar change in the F_1 array presented, which was as far as the tests were carried. These tests were made at the time that other portions of this investigation were in progress, as checks to make certain that the conditions and the results that were found and being obtained in the two main sources of material were not vitiated by the use of only one source of supply, in which something unique might be present and productive of the complications found.

Analysis of the conditions in the habitat of *L. diversa* at Orizaba and *L. signaticollis* at Cuernavaca, as far as data were available, showed during the growing season that essentially the same temperature conditions were found in both habitats, with differences in ranges that were not great. Relative humidity as ordinarily determined showed nothing of interest, while the rainfall at Orizaba

TABLE 7.

Generation.	Date of mating.	Stock used and source.	Average length of ontogeny.	Results and character of breeding tests.	Disposition of products of breeding.
	1907		days.		
I	July 2	L. div. Guadalajara	64 ± 2.1	177 m., 179 f., normal stock.	Mated for gen. II.
I	Do.	L. sig. Atlixco, Morelos	39 ± 3.5	186 m., 185 f., normal stock.	Mated for gen. II.
Test	Do.	L. sig. fem. × m. div.	F_1 sig. 42 ± 2.7 F_1 mid. 56 ± 1.4	44 m., 50 f., sig. 51 m., 49 f., mid. normal types	Not bred further.
II	Oct. 15	L. div. from gen. I as group.	61 ± 2.2	141 m., 146 f., normal stock.	Mated for gen. III.
II	Do.	L. sig. from gen. I as group.	44 ± 4.7	190 m., 189 f., normal stock.	Mated for gen. III.
Test	Do.	L. sig. fem. × m. L. div.	F_1 sig. 46 ± 2 F_1 mid. 58 ± 1.7	27 m., 31 f., sig. 62 m., 60 f., mid. normal types	Not bred further.
Rested from breeding.					
	1908				
III	Do.	L. div. from gen. II as group.	61 ± 2.3	125 m., 126 f., normal stock.	Mated for gen. IV.
III	Jan. 30	L. sig. from gen. II as group.	49 ± 2.7	201 m., 197 f., normal stock.	Mated for gen. IV.
Test	Do.	L. sig. fem. × m. L. div.	F_1 sig. 52 ± 3.1 F_1 div. 60 ± 1.8	11 m., 4 f., sig. 141 m., 137 f., mid. normal types	Not bred further.
IV	Apr. 20	L. div. from gen. III as group.	61 ± 1.6	76 m., 79 f., normal stock.	Mated for gen. V.
IV	Do.	L. sig. from gen. III as group.	56 ± 3.7	92 m., 85 f., normal stock.	Mated for gen. V.
Test	Do.	L. sig. fem. × m. L. div.	F_1 sig. 59 ± 2 F_1 mid. 61 ± 1.9	2 m., 3 f., sig. 171 m., 164 f., mid. normal types	Signaticollis tested in F_2, bred pure; mid. tested gave typical F_1 array.
Rested from breeding.					
V	July 15	L. div. from gen. IV as group.	60 ± 1.4	145 m., 153 f., normal stock.	Mated for gen. VI.
V	Do.	L. sig. from gen. IV as group.	59 ± 2.3	166 m., 159 f., normal stock.	Mated for gen. VI.
Test	Do.	L. sig. fem. × m. L. div.	60 ± 3.2	186 m., 192 f., mid. normal types	10 pair mated at random; all gave typical F_2 array.
VI	Oct. 6	L. div. from gen. V.	60 ± 1.2	110 m., 108 f., normal stock.	Mated for gen. VII.
VI	Do.	L. sig. from gen. V.	60 ± 2.1	170 m., 166 f., normal stock.	Mated for gen. VII.
Test	Do.	L. sig. fem. × m. L. div.	60 ± 1.7	115 m., 111 f., mid. normal type	Tested in F_1.

was greater than at Cuernavaca. The greatest difference in the locations is in the water-relation of the organisms. At Orizaba the relation is one of high

TABLE 8.

Month.	Period.	Atmometer. (Fig. 5), av. daily loss in c. c.*	Free water surface, av. daily loss in c. c.†	Max. loss in c. c.		Min. loss in c. c.	
				Free H_2O.	Atm.	Free H_2O	Atm.
CUERNAVACA, 1905.							
April	20 to 30	107.4	93.2	117.4	164.3	78.4	87.2
May	1 to 10	119.8	108.7	148.1	186.4	92.2	101.4
	11 to 20	112.2	100.4	141.0	204.3	86.1	98.4
	21 to 31	98.7	91.0	117.1	149.5	78.1	84.5
June	1 to 10	92.5	84.1	108.1	146.4	70.4	77.0
	11 to 20	66.4	55.1	80.0	93.4	7.1	19.7
	21 to 30	64.3	54.9	80.0	90.1	24.1	30.4
July	1 to 10	61.7	53.4	81.0	85.1	40.0	42.0
	11 to 20	54.5	47.6	73.0	76.4	21.4	29.3
	21 to 31	51.3	40.1	77.0	84.4	29.1	35.8
Aug.	1 to 10	54.1	41.1	81.0	96.1	31.4	37.7
	11 to 20	64.5	58.7	96.4	114.1	34.1	39.9
	21 to 31	....	....	....	....	....	....
Sept.	1 to 10	76.5	70.1	124.3	177.7	54.4	61.2
	11 to 20	79.4	74.1	121.1	179.1	61.1	50.1
	21 to 30	84.2	81.3	127.1	186.4	65.4	60.3
Oct.	1 to 10	89.8	85.4	129.4	193.5	64.0	69.9
	11 to 20	....	....	....	....	....	....
	21 to 31	87.4	80.1	141.4	201.8	68.1	74.1
Nov.	1 to 10	....	....	....	....	....	....
	11 to 20	....	....	....	....	....	....
ORIZABA, 1906.							
April	20 to 30	77.0	65.2	109.0	141.0	56.2	61.0
May	1 to 10	79.4	74.3	105.0	117.0	54.4	66.1
	11 to 20	87.4	76.1	80.0	101.1	63.2	71.4
	21 to 31	60.1	44.2	61.0	75.4	7.3	11.9
June	1 to 10	43.1	32.1	56.0	61.0	0.0	1.7
	11 to 20	31.1	20.9	41.0	48.0	0.0	0.4
	21 to 30	31.7	24.3	41.4	44.1	0.0	0.0
July	1 to 10	33.5	29.2	42.0	45.9	0.0	0.0
	11 to 20	21.4	17.3	40.4	43.7	0.0	0.0
	21 to 31	20.4	14.1	33.1	40.1	0.0	0.7
Aug.	1 to 10	23.6	26.1	41.1	56.4	0.4	0.9
	11 to 20	31.2	26.4	54.1	61.0	0.0	0.0
	21 to 31	33.4	27.7	54.7	60.7	0.0	0.0
Sept. 1908..	1 to 6	34.5	30.1	56.7	50.4	0.0	0.0
	10 to 18	33.5	31.1	54.7	61.1	0.0	4.1
Nov. 1905..	1 to 5	37.7	34.6	84.1	97.4	0.0	0.0
Jan. 1910..	7 to 14	45.7	39.4	81.1	87.7	0.0	0.0

* Evaporating surface = 800 sq. cm. heavy filter paper hung vertically in open.
† Evaporating surface = 800 sq. cm. in dish, 40 by 20 cm., in open in sun.

water-content in the medium, with relatively low evaporation-rates, hence the prevailing type of plants and animals characteristic of moist habitats in the

tropics, essentially a montane rain-forest, while at Cuernavaca the conditions are those of an upland savannah complex with intense desiccation during nine months of the year, and daily desiccations of varying degrees during the rainy or growing season, from June to September. Records of the evaporation-rates at the two locations have been taken at different times during the progress of this work as opportunity permitted, and some of these are given in table 8. These are taken with the filter-paper atmometer and the free water-surface; the result recorded is the loss of water in cubic centimeters of water evaporated. These and other determinations are shown in the series of curves in figure 6, which show graphically the progress of the season in this respect and the relation of the breeding-periods to this environmental relation. It is at once clear that the complexes are very different in the two locations in this respect, and the rate of evaporation has been found in experiment to be one of the most important agents in the environmental complex; that is, this rate of water-loss from the

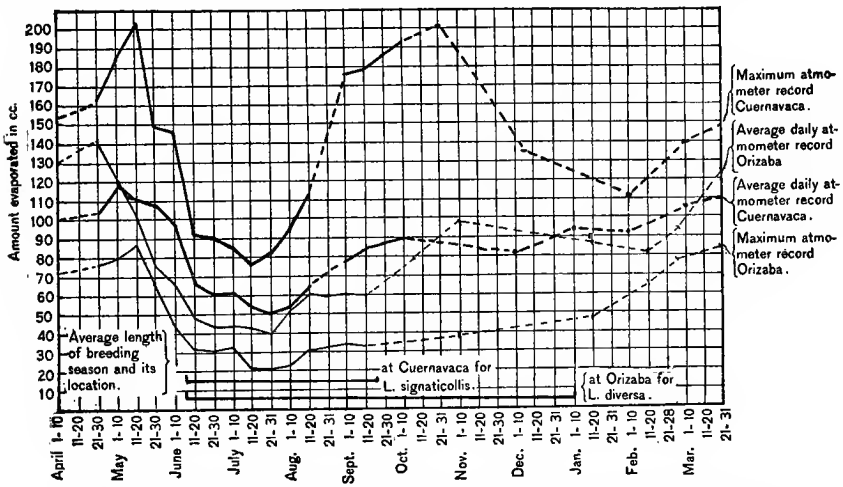


FIG. 6.—Showing differences in evaporation rates in the parental habitat of *L. signaticollis* at Cuernavaca and of *L. diversa* at Orizaba, as determined from various atmometer readings through several years.

surface of the body, which is in general terms comparable to that shown in the atmometer, alters most profoundly the action and rates of reaction in the organism. It will induce changes in the trophisms of the organism most readily, and reversal of the normal activities, as well as serve as an accelerator in ontogeny. Thus the conditions at Cuernavaca are such as to produce rapid ontogenetic development, and the species from that location have high rates of reaction and short ontogenetic cycles, in *signaticollis* about 40 days, while at Orizaba the conditions are such that there is little or no pressure of the environment, and as a result the ontogeny is longer in each of the two annual generations, and there is much overlapping and interbreeding of them.

In the laboratory at Chicago the conditions were a close approach to those found in the Orizaba location, and under these the two species come to have the same rates of development. Each has, therefore, a specific rate of ontogenetic progression that is characteristic of each in its original habitat, but which is

easily changed on introduction into a new habitat, without any change in the characteristics of the organism. It is, as far as I am able to decide, *a condition in the organism, a state of being,* that exists within the mass and is the product in its values of the conditions in the medium in which the organism is living, acting upon and directly modifying the reaction values made possible by the gametic factors of composition, and is in this respect comparable to the *conditions* of the mass that one finds in non-living materials.

The ontogenetic group of developmental reactions are immensely complex, but they have clearly the relation and values in these species, which I have designated the ontogenetic rate determiner (*Ac*), and changes in the reaction value of this agent, induced through changes in the medium, produce in the organic mass changes in the rate of ontogenetic progression, which are measured by the length of the time taken for the completion of growth, from fertilization to the adult condition. The quantitative value of *Ac* in any species I have expressed in terms of elapsed time demanded by the ontogeny of the species, as expressed in days. Thus Ac^{46} or Ac^{60}, simply signifies that in the stock from which the individuals came under the conditions of their culture, this factor had the value of reaction, such that the total series of ontogenetic reactions would be completed, on the average, in 40 or 60 days.

This agent serves as a most important factor in disturbances of the Mendelian reaction in species crosses, and changes the character, as far as visibility is concerned, of many individuals in the population for indefinite periods of time. I have shown that the two species, *L. diversa* and *L. signaticollis,* when crossed under common conditions, and when the values of *Ac* are the same or essentially the same Ac^{60}, give perfect monohybrid reactions in all instances, and without exception showing the production of gametes that are pure for the total system of each of the two species, giving true *signaticollis* and *diversa* as extracted products. On the other hand, it is shown in plate 9 that when the two are crossed where the *Ac* values are different there ensues in F_1 and in following generations a different series of events, in which the fate of certain individuals is permanently altered, and the F_1 reaction *suggests the interpretation* that one of the parents is heterozygous, the other homozygous.

Thus far there has been no exception to the two types of results when the conditions in the medium and the values of *Ac* are those stated, and this represents the condition of the crossing of the two species fresh from nature, or of fresh *L. signaticollis* crossed with the *L. diversa* that has been in the laboratory for any length of time. Also, the same results are produced if *L. signaticollis* is in experiment made to attain the value of Ac^{40} or thereabouts, regardless of what its former rate of ontogeny has been.

In the earlier experiments from 1904 to 1907, the series described were duplicated over and over again, and many tests were made that gave profitable indications for further work. From 1907 to 1914 the reactions found, and represented in plate 9, have been extensively tested and an analysis made of the products. Starting with a series that is easily obtained by the crossing of *L. signaticollis* from Cuernavaca, when freshly introduced into the laboratory, and *L. diversa,* either fresh or in stock, the cross made under the standard conditions of the testing-room gave uniformly 50 per cent of the progeny that were of the typical mid-type, and 50 per cent that were to all appearances *signaticollis*

in type. In F_2 the *signaticollis* type bred true in all instances, the mid-type gave the customary F_2 array in nearly perfect proportions. This obviously heterozygous line shows nothing of any interest or irregularity, no matter how far it is carried and regardless of the conditions of the medium within limits.

In the *signaticollis* line, however, I learned to recognize differences in the population, delicate and not capable of determination except by measurement. In general appearances the population was uniformly *signaticollis* type, bred true, crossed with pure *signaticollis* stock, gave only *signaticollis* types in the progeny, so that one might easily pass the entire population as extracted *signaticollis.* I found that with close attention I could separate three form-types from this F_2 *signaticollis* population. These I first recognized as present in an F_2 population that was *signaticollis* to all outward appearance, of form-types that had the suggestion of *diversa,* of *signaticollis,* and of an *intermediate* type with intermediate conditions in body, form, and aspect.

In 1907 I had measured three random fraternities of this F_2 *signaticollis* type to obtain the form-index. The result of the determinations showed in the F_2 *signaticollis* type population a trimodal polygon, one mode of which was upon that of the *diversa* parent, the other that of *signaticollis,* the third variable and *intermediate,* that of typical heterozygotes. There was not the least doubt of the presence of the three modes in the F_2 fraternities measured, which were supposedly homogeneous. In figure 7 I have shown the results of this determination for the fraternities and the determinations for the two parent species. I also made like determinations of the form-index in a normal heterozygous line and which showed that the conditions in the intermediate groups and in the recognized mid-type was essentially the same. The three fraternities, each the progeny of a single pair of parents, were large—171, 180, and 204—and each showed the same arrangement into a trimodal curve as is shown in the summation of them shown in figure 7. I was thus able also to detect these modal differences of the form-index and so was able to select mates from the two extreme modes that corresponded with the two parent species modes for breeding, and found that the response was immediate to produce in the next generation complete isolation of the two groups, with no more intergrades in form-index than are present in the normal species. These lines were kept breeding on through 1908 and 1909 without change or transgression of the limits of the condition first determined in 1907.

In the latter part of 1908 it was observed that in fraternities belonging to the lower end of the range there was not uncommonly a development of faint, diffuse, irregular pigmentation along the rows of punctation on the elytra (plate 8, figure 11), in position and relations in which pigment is never found in the pure *signaticollis* stock, and has not been made to develop by the extension of the pigment in the pits. It was further found that these conditions in the race were permanent and could by mating likes be intensified to the extent that the elytron presented often a faint brownish tinge extending out on all sides from the system of pits in the surface of the elytron.

These two experiences lead to the hypothesis that the F_1 *signaticollis* race was not *signaticollis* at all, but a masked heterozygous race that had the *diversa* gametic system present but in combination, such that it was not able to produce the normal reaction in the complex to give the usual heterozygote form. This

idea was supported by the ability to separate the F_1 *signaticollis* race into the three groups in F_2, having the modal conditions of form-index respectively of the two parent species, and the known heterozygote, and the presence in the lower portion of this trimodal polygon of the development of dark pigment in an association not known in the original *signaticollis* materials. If the *V* determiner[1] was present, but in new combinations in the gametic complex, it was at least worth the effort to try to recover *V* from this race F_1 of *signaticollis* in appearance, but trimodal in form-indexes in F_2, and derive the *diversa* form from this race as an extracted product by bringing about in some manner the recombination in the gametes of the proper arrangement of gametic agents, which were possibly present but in new arrangements.

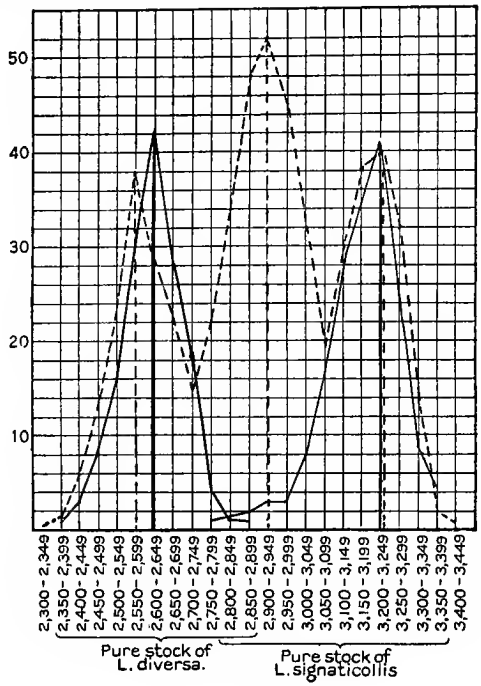


FIG. 7.

To test this point I used only strains of *signaticollis*, so that there might be introduced no traces of the *V* determiner other than that already there. In 1908 and 1909 I made many crosses of this peculiar derivative from the trimodal *signaticollis* race (index mode 2.600-2.649), in which both had the *Ac* determiner with a value of Ac^{60}, with the uniform result that in no fraternity did any trace of the *V* determiner producing the *diversa* striped elytra appear. All of the progeny were *signaticollis* in aspect, but two series of crosses that I had measured showed F_1 heterozygous for form-index, monomodal, and as of the usual F_1 type, and trimodal conditions in F_2. In all 22 crosses were made, with the constant results described, and from these it is certain that the *signaticollis* pure species does not have any agent that matches the *V*, or otherwise

[1] Determiner for elypitcal stripe see tables 2 and 3, pp. 86, 87.

enables it to be recovered from the *signaticollis*-like races that arise in F_1 as an extracted stock.

Another test was made with a *signaticollis* stock that had the *Ac* determiner with a value of Ac^{38}. This was a pure native stock that had been in the laboratory for 15 generations and had from the tenth generation onward been made, by external conditions, to attain the value of Ac^{88}. This was done by placing it for breeding under conditions in which the temperature ranges were those of the native habitat at Cuernavaca, but in which the water-relation was much more rigorous, especially in the evaporation in the daylight hours. Under these conditions two or at the most three generations are all that is needed to attain the full development of the faster value of *Ac*. It was found that when a race of pure *signaticollis*, with the value Ac^{38}, is crossed with the suspected race of *signaticollis*, having the *diversa* form-index, and having the value Ac^{60}, under the conditions used to develop the Ac^{38} value in the pure *signaticollis*, the result is to produce an F_1 array that is *signaticollis* in general aspect, but whose index is intermediate between the two, on the heterozygous mode, and these when inbred showed in F_2 a variable number of *diversa types*, with several others, showing that *V*, the elytral stripe determiner, has been returned to its normal relations, resulting in the production of gametes that are pure *diversa* in all respects, and these when combined with likes produce pure *diversa types*. These were found to breed true in subsequent generations, to be pure *diversa stock* in every way. This reaction became the test upon which this complex and apparently unconformable series was finally elucidated, and is referred to in the remainder of this account of the crosses between the species *signaticollis* and *diversa* as the *test reaction*.

This reaction shows that in the gametes of the extracted *signaticollis* appearing line with *diversa* index there is still present the proper agent, *V*, which, in certain relations of interaction, is able to produce the typical *diversa* elytral pattern; but that in other relations it is not, but produces the condition of a *diversa form*, with the *signaticollis* appearance, and the possible development of pigmentation in irregular spreading areas in connection with the system of elytral punctations. There is present in the elytron the agents needed to produce pigment, and it is constantly active in the production of pigment in the pits that form the elytral punctation series. The *V* determiner is an agent that acts, as far as known, as a pattern-determiner in conjunction with the general pattern-factor of the elytron. For the purposes of the series of crosses between *signaticollis* and *diversa*, only the *Ac* determiner, with its values represented by the exponent accompanying it, the elytral pattern-determiner *Elp*, the determiner for the punctation system (*Pu*) upon the elytron, and the pattern-determiner for elytral longitudinal stripes (*V*). These are agents of specificity in the gamete as is shown by the ability to replace the *Pu* determiner of *signaticollis* by that from some other source, as also the *V* determiner of *diversa* can be replaced by another agent occupying precisely the same relations in this system and productive of its specific type of resultant product. Or, in other operations, the entire group may be dissociated from the gametic complex and transferred entire to a new association. In the figures and plates I have shown these agents with bonds between them, which represent no more than the directions of interaction, or the combinations of action that experience with these

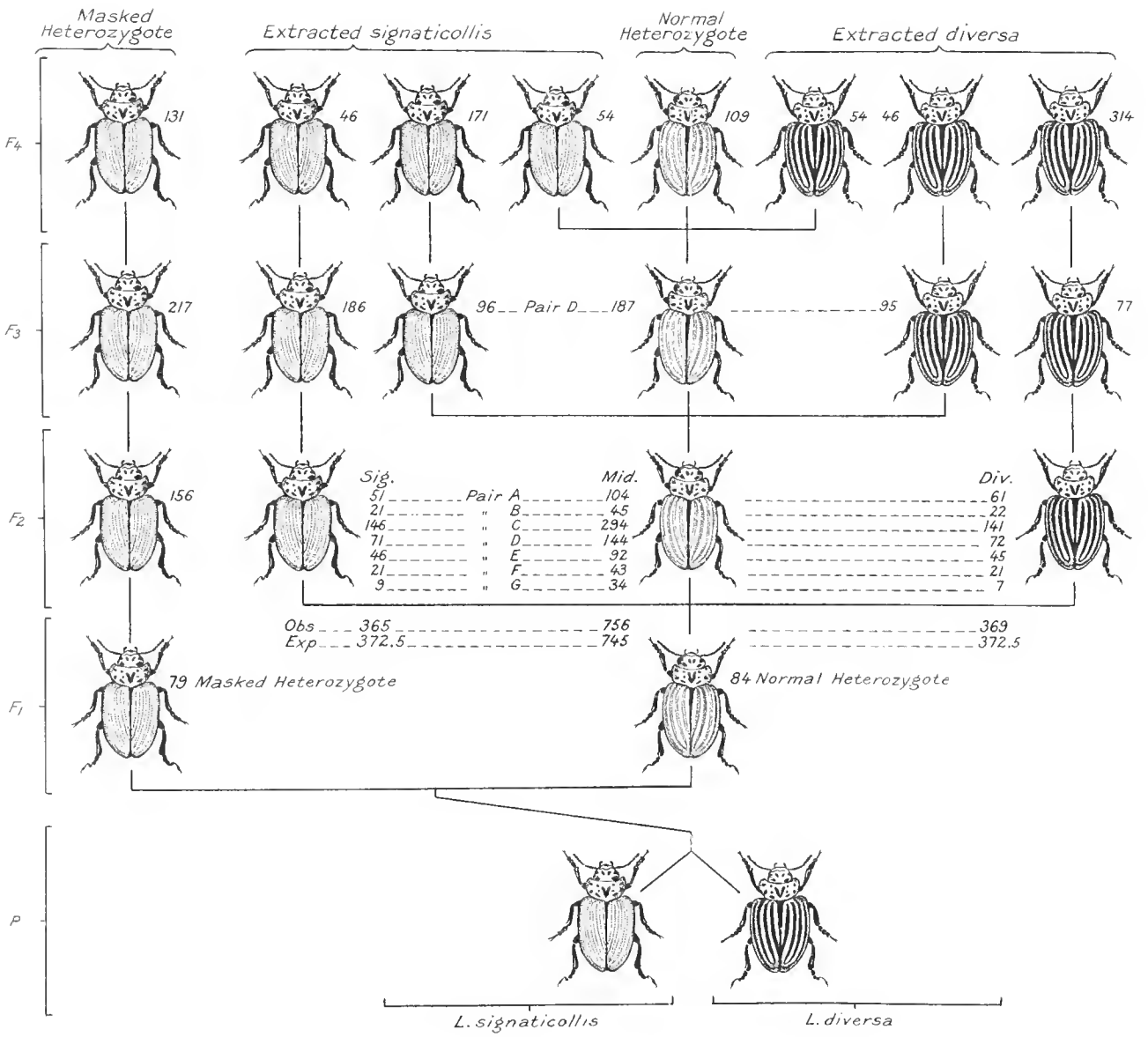
agents shows them to have. At present they are but graphic representations of the associations and interaction of these gametic agents in the production of specific end-results.

The results shown in plate 9, while suggestive of the crossing of one heterozygous and one homozygous parent, are in reality not that at all, but are different, as shown by the test reaction applied to the F_1 fraternities of any of these arrays, which uniformly produces in the following F_2 fraternity, after the test, pure-breeding *diversa* types along with several others, showing that the pure-breeding F_1 *signaticollis* race has in reality in it the *V* determiner, which could not possibly have come from any other source, and that the F_1 *signaticollis* type, while it bred true in aspect, is in reality a masked heterozygote with the *V* determiner inactive, as far as the production of stripes is concerned. This is further made certain if an F_2 fraternity of the questionable *signaticollis* type be separated into three groups by measurements of the form-index and then applying the test to the modal classes of each group.

The test, when it is applied to the portion of the trimodal F_2 polygon having the highest or *signaticollis* index, gives in all following fraternities nothing but *signaticollis,* regardless of what is done to it. The lowest mode, having the *diversa* index, always gives in F_2 pure-breeding extractive types that are *diversa,* while the middle mode also gives in F_2 following the test *diversa* types and others of complex nature. Everything shows clearly that the race of F_1 extracted *signaticollis* is a masked heterozygous strain, a "fixed hybrid," in which the agents that are commonly employed to differentiate the species are inactive. However, biometric testing uniformly shows that F_2 in any such race is trimodal, the extreme modes being that of the parent species form, proving clearly that the F_1 is really heterozygous. The further fact that the race from the lower mode, pure-breeding, and in appearance *signaticollis,* will give the *diversa type* as pure *diversa* following the test reaction; that the other race derived from the higher mode can not be made to do so, shows that *V* is constantly in association with the complex that segregates to produce the lower mode, and is not present in the upper modal group of the F_2 array.

The further observation that in the race derived from the lower mode of the F_2 array, while it is constantly *signaticollis* in its aspect, shows the development of pattern in connection with the punctation system in a way not known anywhere else in all of my materials or in nature, led to the hypothesis that *V* had changed in its relations in the gametic system, such that it was entirely associated with the punctation-determiner, the two acting as a single group. This was shown by the ability to develop, out of the race (plate 8, fig. 11) derived from the lower mode, a derivitive race that showed in all of the members of the fraternities irregular pigment-pattern developing in association with the punctation system where none was known before, and the ability to transfer this condition entire to other series of combinations as in crossing with *L. undecimlineata* or *L. panamensis.* From any such race, either originally or secondarily placed by crossing, the test reaction uniformly gave in the F_2 following the test the pure-breeding extracted *diversa* type.

All of these results show that the real explanation of the apparent inharmonious array shown in plate 9 is in reality due to the forming of new arrangements in the gametic agents. This change consists, as far as determined, in



REACTION IN CROSS OF *L. signaticollis* AND *L. diversa* WHEN VALUES OF THE *Ac* DETERMINER ARE DIFFERENT, BUT CONDITIONS OF MEDIUM ARE NEUTRAL. FIGURES REPRESENT THE FRATERNITIES OBTAINED IN A SINGLE LINE OF CULTURES.

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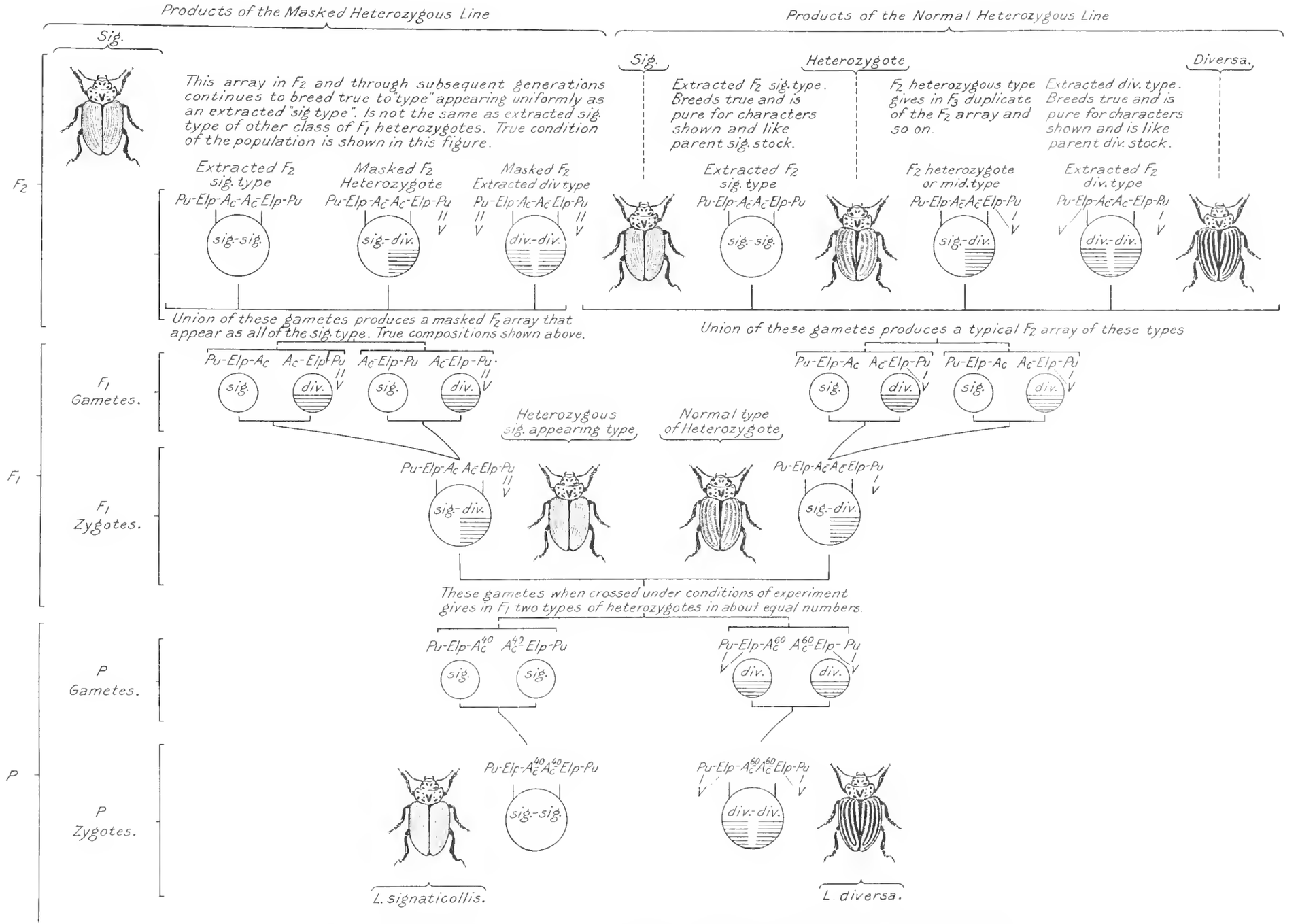
Products of the Masked Heterozygous Line
Products of the Normal Heterozygous Line
Sig.
Sig.
Heterozygote
Diversa.
This array in F2 and through subsequent generations continues to breed true to "type" appearing uniformly as an extracted "sig type". Is not the same as extracted sig. type of other class of F1 heterozygotes. True condition of the population is shown in this figure.
Extracted F2 sig. type
Pu-Elp-Ac-Ac-Elp-Pu
sig.-sig.
Masked F2 Heterozygote
Pu-Elp-Ac-Ac-Elp-Pu
sig.-div.
Masked F2 Extracted div type
Pu-Elp-Ac-Ac-Elp-Pu
div.-div.
Extracted F2 sig. type. Breeds true and is pure for characters shown and like parent sig. stock.
Extracted F2 sig. type
Pu-Elp-Ac-Ac-Elp-Pu
sig.-sig.
F2 heterozygous type gives in F3 duplicate of the F2 array and so on.
F2 heterozygote or mid. type
Pu-Elp-Ac-Ac-Elp-Pu
sig.-div.
Extracted div. type. Breeds true and is pure for characters shown and is like parent div. stock.
Extracted F2 div. type
Pu-Elp-Ac-Ac-Elp-Pu
div.-div.
F2
Union of these gametes produces a masked F2 array that appear as all of the sig. type. True compositions shown above.
Union of these gametes produces a typical F2 array of these types
F1 Gametes.
Pu-Elp-Ac
Ac-Elp-Pu
sig.
div.
F1
Heterozygous sig. appearing type
Normal type of Heterozygote
F1 Zygotes.
Pu-Elp-Ac Ac-Elp-Pu
sig.-div.
These gametes when crossed under conditions of experiment gives in F1 two types of heterozygotes in about equal numbers.
P Gametes.
Pu-Elp-Ac40
Ac43-Elp-Pu
Pu-Elp-Ac60 Ac60-Elp-Pu
P
P Zygotes.
Pu-Elp-Ac40Ac40-Elp-Pu
sig.-sig.
Pu-Elp-Ac60Ac60-Elp-Pu
div.-div.
L. signaticollis.
L. diversa.

SCHEMATIC REPRESENTATION OF RESULTS OBTAINED IN CROSSING *L. signaticollis* AND *L. diversa* UNDER THE SAME CONDITIONS AS SHOWN IN PLATE 9, BUT REPRESENTING BEHAVIOR OF GAMETES AND CHARACTER OF COMBINATIONS WHICH HAVE BEEN SHOWN TO RESULT.

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the formation of the determiners for striping and punctation into a closely bound association, which thenceforward acts homogeneously to produce no trace of stripes in subsequent generations, owing to the lack of interaction of *V* with *Elp*. What it is that changes this arrangement in the system I do not know, except that it occurs only when *Ac* has the relative values of 60 and 40, and under conditions of the medium as given. It would at present be useless to speculate upon what the actual change is in the material constitution of the gamete. The entire complex series of crosses between these two species thus becomes intelligible and in accord with the principles of factorial constitution and operation, capable of elucidation without making assumptions of agents either internal or external, or relations, associations, or results that can not be put to test in one way or another. The entire series shown in plate 9 and all of its consequences works out as is shown in the following analysis of the operations as outlined above.

In plate 10 is shown the parental conditions and the events in the F_1 and F_2 of an array like that shown in plate 9. The complex under which this cross has in general been made is that of the breeding quarters, as follows:

TABLE 8A.

	Temperature.	Relative humidity.
Daily average	80° ± 5°	75° ± 5°
Night average	75° ± 8°	75° ± 5°

Wind movement continuous and causing a complete change in the medium at least once in 30 seconds, giving an evaporation-rate higher than that in the habitat of *diversa* in the growing-season, lower than that of *signaticollis*.

The two parent stocks with the *Ac* values of *diversa* (Ac^{60}) and *signaticollis* (Ac^{40}), when crossed under the conditions given, produce uniformly the stated results in about equal ratio of 1:1. This ratio, however, is only a product of the special complex, both within and without.

The F_1 types both produce the same array of gametes, except that in the *masked heterozygote*, the *V* determiner in the *diversa* gametes has in this series formed in the interactions in F_1 the new association shown in the graphic representation in plate 10. From this point onwards, *Pu-V* exists as a group, acting as a single agent in the gametic operations. Each of the F_1 heterozygotes forms two classes of gametes, giving in F_2 the typical array of three classes in the ratio of 1:2:1, in one set readily visible, in the other only detectable by biometric measurement and the test reaction. In both the extracted types breed true. In plate 11 is shown the continuation of these true-breeding lines, from F_2 onward through F_4, F_5, and F_6, the mid-type or heterozygous mode not being continued, as it duplicates repeatedly the F_2 behavior. In F_5 is shown, in graphic form, the application of the test reaction to the two lines. When crossed with the line derived from the F_2 higher or *signaticollis* mode, nothing happens, and the reason is here shown in the gametic compositions that enter into this combination, no elements but pure *signaticollis* entering; consequently none other is brought out. In the other line, the cross of an F_4 extractive from

the lower or *diversa* mode produces an F_8 zygote that is heterozygous, but appears as *signaticollis* in type.

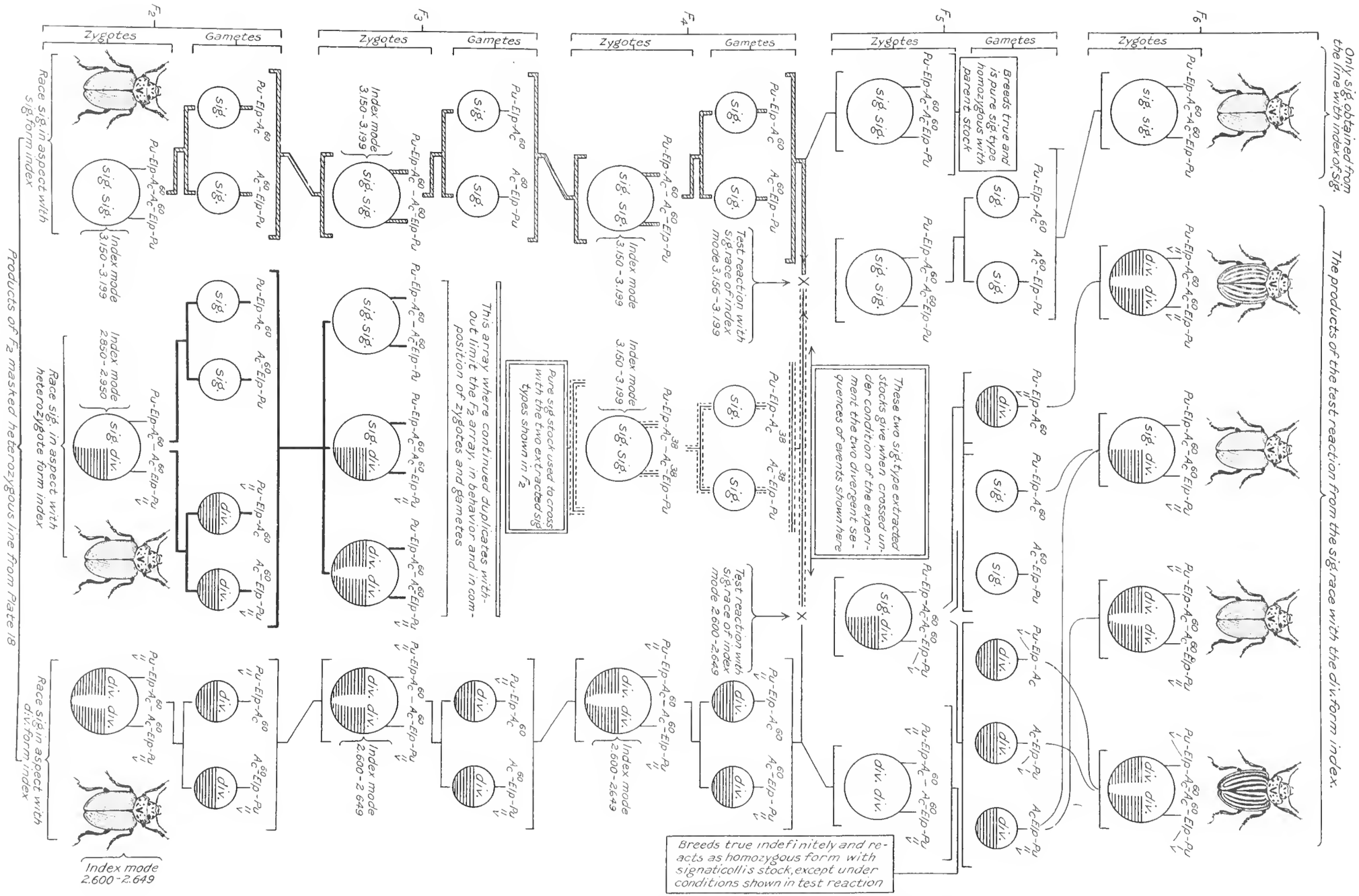
The *Ac* values of all the lines, and indeed after F_1 of the original cross, are Ac^{60}, all trace of the value Ac^{40} having gone from the lines. When the cross is made between the test *signaticollis* at the value of Ac^{38} or Ac^{40}, under the general conditions of the experiment as far as temperature and relative humidity are concerned, there always appear, following the test, *diversa* types in the F_2 fraternities. The numbers present are not constant, and it has been found that the highest number appears when there is little or no wind-movement, hence low evaporation; that the least frequent comes with wind-movement producing rapid evaporation. Plate 11 shows only the fact that the *diversa* type comes out as the result of the test reaction.

In plate 12 is shown the full array that comes in the test reaction when made between pure *signaticollis* with Ac^{40} and the tested form from the lower mode with Ac^{60}, under the conditions given. The full F_2 array shows the following types:

(*a*) *diversa,* a type in all respects *diversa* that breeds true.
(*b*) *mid-type a,* that is, in all respects a heterozygote in appearance and in reaction between the two species.
(*c*) *mid-type b,* of lighter appearance than the preceding, heterozygous, and giving in F_2: 1 *diversa,* 3 *signaticollis*-appearing types within close limits to the expected ratios.
(*d*) *mid-type c,* different from the other two, and in F_2 giving 1 *diversa,* 2 *mid-type c,* 1 new *signaticollis* type, also in close realizations to the expected frequency.
(*e*) *signaticollis type,* that is, the extracted lower mode race, breeding true.
(*f*) *signaticollis type,* in appearance pure *signaticollis,* in reality heterozygous, giving in F_2 all *signaticollis,* but arrayed in trimodal polygons, the modes being on the index-modes of the parent species.
(*g*) *signaticollis type,* that is, pure, true-breeding *signaticollis.*
(*h*) *signaticollis type,* that is, heterozygous, for a new arrangement that comes out in the test.
(*i*) *signaticollis type,* that appears as *signaticollis,* but is really heterozygous for the two arrangements *Pu-V* and *Elp-V.*
(*j*) *signaticollis type,* that breeds true, is homozygous, for the association of the *V* determiner with the *Elp* factor producing general pigmentation in the elytron, and by inbreeding almost black individuals can be obtained.

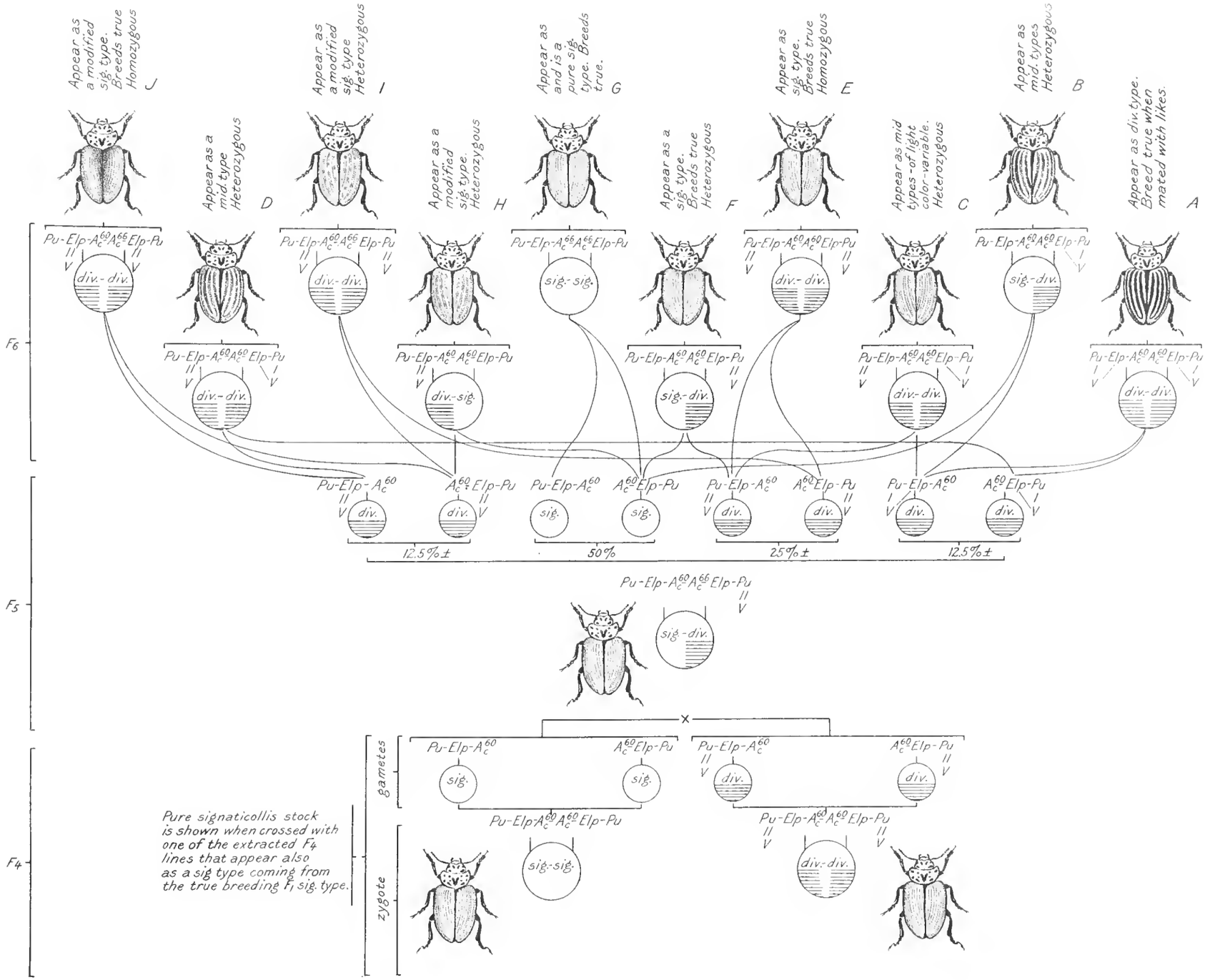
As far as experience goes, the F_1 test hybrid produces two main classes of gametes, 50 per cent that are *signaticollis* and 50 per cent *diversa.* The *signaticollis* gametes in all tests are apparently pure and unaltered, but the *diversa* gametes show with changing conditions recombinations between the three essential agents involved. The condition shown in plate 12 is that realized most frequently under the conditions of experiment, with three classes of *diversa* gametes present. The relative number of these is apparently entirely the combined product of the external conditions, the *Ac* values in the two stocks interacting in the gametogenesis or at some other time in the life of F_1.

This in general is the result of the test reaction, revealing as it does the existence of the *V* determiner and its restoration in some of the gametes to its normal relations, resulting in the recovery of the lost *diversa* type. Of interest


F2
F3
F4
F5
F6
Zygotes
Gametes
Only sig. obtained from the line with index of 3.199
The products of the test reaction from the sig. race with the div. form index.
Products of F2 masked heterozygous line from Plate 18
Race sig. in aspect with sig. form index
Index mode 3.150 - 3.199
Race sig. in aspect with heterozygote form index
Index mode 2.850 - 2.950
Index mode 2.600 - 2.649
Race sig. in aspect with div. form index
Test reaction with sig. race of index mode 3.156 - 3.199
Test reaction with sig. race of index mode 2.600 - 2.649
Pure sig. stock used to cross with the two extracted sig. types shown in F2
This array where continued duplicates without limit the F2 array, in behavior and in composition of zygotes and gametes
These two sig. type extracted stocks give when crossed under condition of the experiment the two divergent sequences of events shown here
Breeds true and is pure sig. type homozygous with parent stock
Breeds true indefinitely and reacts as homozygous form with signaticollis stock, except under conditions shown in test reaction

A DIAGRAMMATIC REPRESENTATION OF SOME RESULTS, THE FIRST PART OF WHICH ARE SHOWN IN PLATE 10. THIS PLATE SHOWS THE CONTINUED BREEDING OF CERTAIN LINES STARTED IN PLATE 10 AND THEIR CHARACTERS AS REGARDS GAMETES AND PLACING OF CERTAIN FACTORS. IN F_6 IN THIS PLATE IS SHOWN THE APPLICATION OF THE TEST DESCRIBED IN THE TEXT TO SUSPECTED STOCKS OF THIS KIND AND IN F_6 SOME OF THE RESULTS HAVE COME FROM THE APPLICATION OF THIS TEST TO THESE SUPPRESSED HETEROZYGOTIC TYPES.

JULIUS BIEN LITH., N. Y.



To show in detail the Complication of the Test Reaction described in text.

in this test series is the formation of a new association of the *V* determiner, giving a new race in the series that breeds true.

In the further breeding the new race (*J*) that comes out in the test and its testing with the same reaction show that it is in no wise an exception to the entire series, but that the test will, under the conditions of experiment, produce the dissociation of the agents, restoring them in some of the gametes to their original relations, and giving *diversa* as the result in lines in which it had not been present for generations. This dissociation, in this instance, is easy to accomplish if the temperature and humidity are increased above that previously used and if the evaporation rate is low.

There results from this testing and other operations two races that in appearance are *signaticollis,* in reality are *diversa,* with the *V* determiner in different positions in the gametic system, and that breed true without limit.

This series of observations and experiments with crosses between two species under conditions that give the reaction shown in plate 9 are interesting and suggestive of many important considerations. In that portion of the F_1 population that was *signaticollis*-like in aspect there were possibilities for misinterpretation and wrong conclusions and operations. The fact that it bred true, so that in ordinary operations it would have passed as a pure extracted race of *signaticollis,* that had come out as the result of one or the other of the parents being " heterozygous " for some complement or factor present in homozygous condition in the other, might easily lead to confusion, and the further test crossings would have been interpreted as mutations in the extracted line. If the operation had taken place in nature and had been unknown, and the stock had come into our hands in the laboratory, the conclusion would be drawn that it had " mutated," giving new " elementary species " or races.

Aside from the potentialities for mistakes in the laboratory, the series is suggestive of important rôles in nature, both in the matter of rendering species in nature complex and also in the rôle of serving as an actual motive force in evolution. It is not difficult to picture a series of events in nature, such that introduced forms in crossing would, in the manner shown here, gametically change the nature of the resident form without in any way altering its phenotypical aspect, so that it would pass as one form, " variable " perhaps, which at some future time might mutate, producing quite new characteristics, which were in reality only the reconstitution of an arrangement that had previously formed a portion of the attributes of some intercrossed species. An apparently pure *signaticollis* race, when crossed with another of known purity under the conditions internal and external that have been stated, showed in the test in F_2 an array of 10 types in differing proportions. Some of these 10 are pure-breeding when mated with likes and remain constant, even though they may be heterozygous. Others are pure, as far as tests can determine, and others are heterozygotes in the ordinary sense. The array might in nature well be the basis for the origin of new groups or species.

In many respects the action of the *signaticollis* race that comes out in F_1 reminds one of the fixed hybrids that are often described. I have had a number of instances in my cultures of fixed pure-breeding heterozygous races, and all appear as fixed hybrids in the ordinary tests that are applied. The fact that the form *comes true* in breeding may be of importance, but it is not a reliable

criterion that it is either homozygous in action or in composition, and its apparent homozygous action may well be the product of inability to make manifest the conspicuous differentiating characters, and so the series is passed on as pure-breeding extractives, when in reality the thorough testing of such races is necessary to determine their true characters. In the great majority of the instances I have had to deal with, the usual tests of present neo-Mendelian operations are not sufficient.

The most important internal agent is the *Ac* determiner and its values, and the most important external one is the water-relation, as measured in terms of evaporation from the body. These animals obtain water only from their food and as hygroscopic water that is absorbed from the air through respiration. They are extremely sensitive to changes in the water-relation, and it is suggestive that a relation has been found between the water-loss, the determiner *Ac*, and the recombination of the *V* determiner in the operations of F_1. The modifiability of the internal mechanism represented by the *Ac* determiner, its rapid change to a balance with the conditions of the medium, and its readiness to shift its values within limits, suggests most strongly not any substantative thing in the organism, but rather a general state of the entire mass of the system in the relations of its component atomic or molecular elements; that is, productive of rapidity of reactions in one instance or their prolongation in others. Within any species or group of species this agent is capable of change only within limits, and I have not in any instance been able to modify it beyond those. Thus, in *L. signaticollis,* 29 days is the absolutely low limit, and one that can not be maintained, while 37 to 40 days is the lowest that can be maintained with any success. The upper limit is 63 days as the condition that can be held in the strain for purposes of work. In *L. diversa,* the lower limit is about 50 days, the upper 70 days or thereabouts. Even though plastic in its nature, this *general condition* in the mass of the system is specific within limits for that mass, and the specificity of the condition segregates with the mass of each gamete with certainty and distinctness. None of the extracted *diversa* gametes or zygotes in these experiments take on the rate found in *signaticollis* at its more rapid limit, nor is *signaticollis* made to react as slowly as can *diversa,* but in the cultures both may and do react perfectly within the mid-limits of their ranges, and when the two are reacting in these values, crossing of the two species is always accompanied with the most regular and stereotyped monohybrid Mendelian reaction. Divergence in the rates of action of this agent in the gametes is productive of the changed arrangement in the agents that are productive of elytral characters, although why it should be these and not others it is not possible to state. It may well be true that there are others that are not observed that are changed, and that added investigation will disclose them.

Precisely the same series of events occur if the cross is made in the other direction, *diversa* female and *signaticollis* male, so there is no discovered relation of the action to sex, or sex-linking.

Decidedly interesting relations of this reaction to the external conditions in the medium have been developed as the outcome of this series of experiments and its analysis thus far. These experiments show a relation of the operation of the internal agents to the conditions in the medium that is most suggestive.

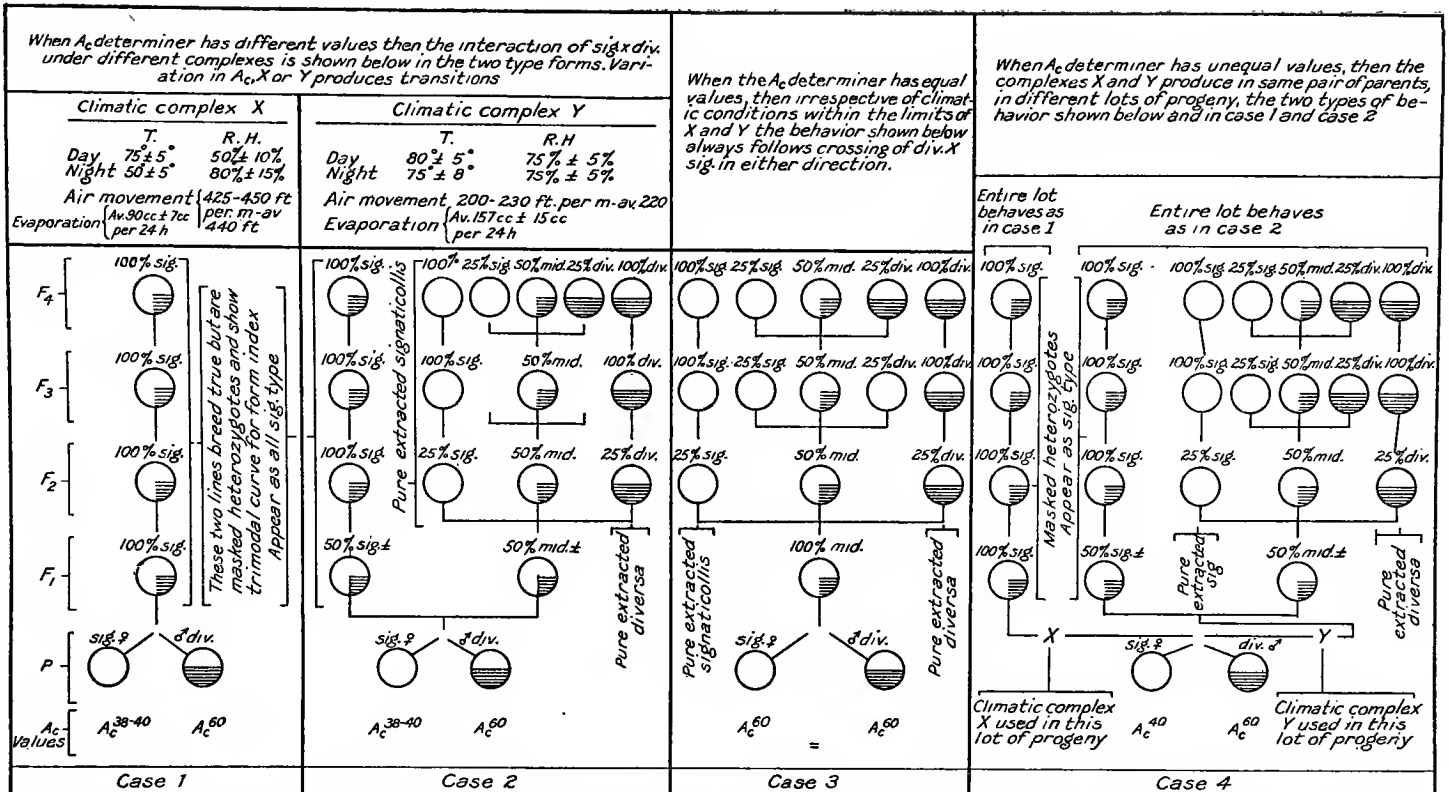

When Ac determiner has different values then the interaction of sig x div. under different complexes is shown below in the two type forms. Variation in Ac, X or Y produces transitions
Climatic complex X
T.
R.H.
Day 75° ± 5°
50% ± 10%
Night 50° ± 5°
80% ± 15%
Air movement 425-450 ft per m-av
Evaporation Av. 90 cc ± 7cc per 24 h
440 ft
Climatic complex Y
T.
R.H
Day 80° ± 5°
75% ± 5%
Night 75° ± 8°
75% ± 5%
Air movement 200-230 ft. per m-av. 220
Evaporation Av. 157 cc ± 15 cc per 24 h
When the Ac determiner has equal values, then irrespective of climatic conditions within the limits of X and Y the behavior shown below always follows crossing of div. X sig. in either direction.
When Ac determiner has unequal values, then the complexes X and Y produce in same pair of parents, in different lots of progeny, the two types of behavior shown below and in case 1 and case 2
Entire lot behaves as in case 1
Entire lot behaves as in case 2
F4
F3
F2
F1
P
Ac Values
100% sig.
These two lines breed true but are masked heterozygotes and show trimodal curve for form index
Appear as all sig. type
sig. ♀
♂ div.
Ac38-40
Ac60
Pure extracted signaticollis
50% mid.
25% sig.
25% div.
50% sig.±
50% mid.±
Pure extracted diversa
100% mid.
Masked heterozygotes Appear as sig. type
Pure extracted sig
X
Y
div. ♂
Climatic complex X used in this lot of progeny
Ac40
Climatic complex Y used in this lot of progeny
=
Case 1
Case 2
Case 3
Case 4

FIG. 8. —Schematic representation of results obtained in crossing *L. signaticollis* and *L. diversa* with the A values of different amounts under different conditions of the medium. This figure in schematic fashion visualizes the results obtained in this series of experiments so far as they relate to production of diversity in the crossing of species. Cases 1, 2, 3, and 4 are adequately explained in the text to which reference should be made. The clear circles designate *signaticollis*, the cross-lined circles *diversa*, and those in which the lower two quadrants are one cross-lined and one blank represent heterozygous conditions.

In figure 8 I have put in condensed form a schematic presentation of the general results that have been obtained in the study of the rôle of external conditions in modifying the reactions in crossing. In these experiments there are two important agents—the value of *Ac* and the composition of the climatic conditions in the medium; but since the value of *Ac* is the direct product of the conditions and duration of the race in the medium, the external complex plays the major rôle in the production of changes in the Mendelian reaction. I have used two sets of conditions, as follows:

TABLE 8B.

	Climatic complex X.			Climatic complex Y.	
	Temperature.	Relative humidity.		Temperature.	Relative humidity.
	° F.	*Per cent.*		° F.	*Per cent.*
Day	75 ± 5	50 ± 10	Day	90 ± 5	75 ± 5
Night	50 ± 5	80 ± 15	Night	75 ± 8	75 ± 5
Air-movement, 425 to 450 ft. per min.; av., 440. Evaporation, loss in cm., av. 84.6 cm. per 24 hrs.			Air-movement, 200 to 230 ft. per min.; av., 220. Evaporation, loss in cm., av. 41.7 cm. per 24 hrs.		

With stocks of known standard reactions, when crossed, under the conditions of climatic complex *X*, with the *Ac* values 40 and 60 respectively, the uniform result is the production in F_1 of all *signaticollis* type, and these in F_2 breed true; but the polygon of all fraternities that have been tested are trimodal, whereas the line continues to appear to breed true as a *signaticollis*-appearing type, as shown in case 1, figure 8. Under these conditions the desiccation is intense and the result is to accelerate ontogeny, so that the reaction in F_1 is to alter the arrangement in all of the gametes, and even in the F_1 heterozygotes, so that they are in aspect *signaticollis*. This is a striking result, and although the result breeds true in ordinary terms, it is truly in all instances thus far seen heterozygous, as may be demonstrated by biometric analysis of the individual fraternities, or by application of the test reaction. Both tests are equally good, but the biometric test must not be applied to more than the members of a single fraternity if the results are to be clear and useful. In table 9 I have given the

TABLE 9.

Date.	P.	All signaticollis.												Total.
		F_1		F_2		F_3		F_4		F_5		F_6		
		M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	
Apr. 1904	Sig. m. A^{38} × div. fem. A^{61}.....	76	95	151	150	199	204	42	44	74	77	151	150	1898
July 1904	Fem. sig. A^{40} × m. div. A^{61}.....	49	46	214	226	196	199	....	....	....	....	....	....	930
	M. sig. A^{40} × fem. div. A^{61}.....	55	61	314	327	46	54	....	....	....	....	....	....	857
Mar. 1907	Fem. sig. A^{41} × m. div. A^{58}.....	14	17	56	51	121	127	46	50	92	84	....	....	658
Apr. 1907	Fem. sig. A^{40} × m. div. A^{61}.....	27	24	156	154	44	51	....	....	....	....	....	....	453
Apr. 1907	M. sig. A^{42} × fem. div. A^{59}.....	74	86	46	54	....	....	....	....	....	....	....	....	260
Aug. 1907	Fem. sig. A^{40} × m. div. A^{60}.....	38	39	86	92	146	137	87	92	....	....	....	....	718
June 1909	Fem. sig. A^{41} × m. div. A^{60}.....	12	9	186	197	....	....	....	....	....	....	....	....	404
	M. sig. A^{41} × fem. div. A^{60}.....	44	42	145	156	....	....	....	....	....	....	....	....	387
Aug. 1910	Fem. sig. A^{37} × m. div. A^{62}.....	77	74	27	31	214	226	56	54	92	87	....	....	938
	M. sig. A^{37} × fem. div. A^{62}.....	73	61	29	41	276	281	171	192	85	106	....	....	1315
Total obs.	.....	....	....	....	....	....	....	....	....	....	....	....	....	8313

TABLE 10.

Date.	P	F_1	F_2	F_3	F_4	F_5
Apr., 1904..	Sig. fem. $A^{58} \times$ div. m. A^{61}	49 sig.	186 sig.	79 sig.	51 sig.	144 sig.
			104 sig.	139 sig.	154 sig.	276 sig.
				92 sig.	246 sig.	
		53 mid.	216 mid.	189 mid.	give same array	as F_3.
				90 div.	287 div.	
			107 div.	256 div.	146 div.	483 div.
Mar., 1907..	Div. m. $A^{41} \times$ sig. fem. A^{58}	121 sig.	439 sig.	385 sig.	493 sig.	
			110 sig.	431 sig.	425 sig.	241 sig.
				92 sig.	86 sig.	
		118 mid.	221 mid.	190 mid.		
				85 div.	156 div.	
			107 div.	456 div.	519 div.	431 div.
	Sig. fem. $A^{41} \times$ div. m. A^{58}	49 sig.	531 sig.	462 sig.	485 sig.	277 sig.
			321 sig.	425 sig.	122 sig.	
		53 mid.	658 mid.			
			330 div.	686 div.	542 div.	
June, 1909.	Sig. fem. $A^{41} \times$ div. m. A^{60}	27 sig.	192 sig.	321 sig.	292 sig.	451 sig.
			156 sig.	96 sig.	84 sig.	
				461 sig.		
		35 mid.	321 mid.	942 mid.		
				455 div.		
			160 div.	321 div.	76 div.	214 div.
June, 1909.	Div. m. $A^{41} \times$ sig. fem. A^{60}...	96 sig.	83 sig.	77 sig.	141 sig.	135 sig.
			227 sig.	761 sig.	328 sig.	
				426 sig.		
		111 mid.	460 mid.	863 mid.		
				424 div.		
			230 div.	181 div.	124 div.	93 div.

data that has been derived from experiments of this character extending from 1904 to 1910 and carried in all far enough to make certain of the result. In the 11 tests of this sort that are given, the uniform result is the entire failure of these heterozygous types to split up in any of the 8313 individuals in the record. All of the lines have been tested at one time or another, either biometrically or by the test reaction, and all of them showed unmistakable heterozygous conditions in the fraternities, either in the trimodal polygon, with the modes on the modes of the parent species, or the recovery of the *diversa* form from the *signaticollis* strain in the manner described in the preceding pages.

When the same materials are crossed under the conditions of the climatic complex *Y*, the stereotyped result is the production in F_1 of the *signaticollis type* and *mid-type* in about equal numbers, as is shown in plate 10, and in diagram in figure 8, case 2. In table 10 is given the data from 5 experiments, showing the uniformity of behavior in case 2, and aside from the change in the reactions of the masked heterozygotes, the remainder of the population reacts in perfectly regular manner.

The behavior and modifications of reactions shown in figure 8, cases 1 and 2, only follow when the values of *Ac* are at about 40 in *signaticollis* and 60 in *diversa*. As the two values approach, the reactions change, and many transition stages have been found between the two types represented, so that in the end the reaction is that shown in case 3, in figure 8, in which the reactions are normal monohybrid in all respects. This type of reaction, shown in case 3, is of interest in that it is not disturbed by the conditions of the medium, even when more extreme than those in complexes *X* and *Y*. For example, stocks of these two species have been sent from the laboratory at Chicago to the plant at the Desert Laboratory at Tucson, where the conditions given them were desert surroundings; nevertheless, there has not been any change in the production of the F_1 of the usual mid-type array, with F_2 showing the three usual types. In the same manner, in the experimental conditions in the laboratory, the conditions of the medium do not, within limits, change the reaction of the crossing of the two species in any respect when the *Ac* values are the same. In table 11 are given some arrays of the crossing of these two when the *Ac* values are the same.

TABLE 11.

Date.	P.	F_1	F_2	F_3	F_4	F_5
May, 1907.	Sig. fem. A^{60} × div. m. A^{60}.	93 mid. ...	256 sig...	181 sig...	93 sig...	107 sig.
				177 sig...	46 sig...	
			516 mid..	469 mid	122 sig...	64 sig.
					252 mid..	131 mid.
				182 div.	124 div..	63 div.
			260 div...	— div..	— div...	
Do. ...	Sig. m. A^{60} × div. fem. A^{60}.	107 mid. ...	576 sig...	72 sig...	46 sig...	93 sig.
				90 sig...	121 sig...	73 sig.
			1158 mid..	181 sig. .	201 sig...	
					404 mid.	
					198 div..	
				91 div. .	310 div...	214 div.
			581 div...	291 div...	443 div..	106 div.

An interesting complication is introduced into the series by the reactions shown in figure 8, case 4, in which the output from a single pair of parents may be altered, if the parents have the proper *Ac* values, by the application of the climatic complexes *X* and *Y* to the parents at the time of fertilization of the gametes and in the early stages of development. In the figure is shown the process of production in the same pair of parents of the two discordant reactions of cases 1 and 2 in the progeny, merely by the use of the proper climatic complexes at the appropriate moment. In this there is nothing that is not in harmony with the other findings in this series of experiments, but it checks some of the results of the other cases, showing that the differences produced are really the product of the differing *Ac* values, the conditions of the medium, and the nature of the two gametic systems. In all, it is purely a dynamic product of the interaction of agents, within and without the organic mass, and in no wise anything but purely physical in conception and operation. In the laboratory the order in this type 4 can be varied so that the rôle of any possible age affects strength of parents, "orthogenetic," "ontogenetic progressive," gametic change, and kindred "interpretations" may be checked and effectually eliminated. No trace of the operation of any of these supposed agencies has been found. In table 12 is given data from four experiments of this sort, showing the average results that are obtained from type 4 reactions.

I have presented in this digest of the crossings of these two species the results of much more work than appears upon its surface. From 1907 to 1914 the series has been in continuous operations, with a minimum of 4 generations per year, and more than 100 matings per year that were productive of progeny. Throughout, the fraternities have been strong and often have been purposely limited in size to save labor and food, so that the total numbers might easily have been far larger than they have been, but no purpose would probably have been served by this increase in mere numbers. I have never had the total numbers compiled that have passed through our hands in this study, but breeding not less than 100 fraternities per year, with on the average of 50 progeny per pair, gives an estimated number of about 25,000 that have in one way or another been consumed in this investigation. Many of these are either routine tests of the material or tests of the different products of experiment, and have been used merely as indicators of the composition in the manner shown. The counts of a considerable part of these fraternities have been made by Mr. Kuehnel or others, and I have in many and in all critical instances checked the determinations, suspicious cases being bred out and otherwise tested to decide their nature. The earlier series from 1904 to 1907 are regarded as preliminary tests and do not enter into this account to any extent.

The rôle of a reaction of this kind in a state of nature is obviously important. It is not difficult to discover in nature, or to conceive of conditions and accidents producing exactly the array that is shown in case 1, where the result would be, while in all aspects and classification a *signaticollis* race in the location, one with potentiality for change, *per saltum,* on the realization of the proper agent entering into the history of the race, producing *diversa* or some or all of the other types described. In the laboratory, if perchance the reaction seen in case 1 were all that had been seen, the immediate conclusion would be that the production of a fixed hybrid that breeds true for generations had been accomplished. Case 2 in nature might also be a potent agent in evo-

TABLE 12.

Date.	P.	F_1	F_2	F_3	F_4	F_5
Aug., 1907..	Sig. fem. $A^{40} \times$ div. m. A^{60}	1. x, 193 sig.....	246 sig.	122 sig.	131 sig.	277 sig.
		2. y, 88 sig.....	187 sig.	144 sig.	286 sig.	164 sig.
		2. y, 94 mid. ..	146 sig.	171 sig.		
				121 sig.		
			302 mid.	251 mid.		
				125 div.		
			151 div.	412 div.	134 div.	214 div.
	Sig. m. $A^{40} \times$ div. fem. A^{60}	1. x, 47 sig.....	121 sig.	146 sig.	139 sig.	277 sig.
		2. y, 66 sig. ...	125 sig.	496 sig.	381 sig.	47 sig.
		2. y, 64 mid....	140 sig.	224 sig.	146 sig.	
				66 sig.		
			282 mid.	123 mid.		
				65 div.		
			139 div.	129 div.	171 div.	418 div.
June, 1909.	Sig. fem. $A^{41} \times$ div. m. A^{60}	1. x, 49 sig.....	141 sig.	138 sig.	291 sig.	
		2. y, 92 sig. ...	181 sig.	461 sig.		
		2. y, 87 mid....	207 sig.	92 sig.		
			418 mid.			
			209 div.	214 div.	96 div.	
	Sig. m. $A^{41} \times$ div. fem. A^{60}	1. x, 57 sig.....	149 sig.	171 sig.	183 sig.	214 sig.
		2. y, 26 sig.....	46 sig.	92 sig.	31 sig.	122 sig.
		2. y, 26 mid....	66 sig.	46 sig.	164 div.	
				214 sig.		
			130 mid.	430 mid.		
				215 div.		
			65 div.	417 div.	121 div.	64 div.

lution; and in the laboratory, if it alone had been seen, an easy interpretation would have been that one parent was homozygous, the other heterozygous, for some character or agent. Case 3 and case 4 show the remainder of the relations and the complexity of the actual series.

The essential agent in the entire series beyond question is the *Ac* determiner, and its changes with the change of environment of the stocks, its capacity to be altered rapidly in experiment, and the limits of change that it shows in both of the species. It is clearly, in its operation to the conditions in the medium, closely and accurately balanced, and especially to the relation of water-loss in the organism and the consequent change in the relations and associations of the agent productive of elytral color marks and pattern. That the *Ac* determiner is a specific agent in the gametic complex is shown by its limits in any one species that are specific for that, and by the fact that it is not capable of replacement in the complex by the corresponding agent from another source, thus giving in materials that are visibly the same, different *Ac* value ranges and different lengths of ontogeny, the present measure of the *Ac* determiners reaction value. Every indication regarding the nature of the *Ac* agent tends to show that it is a property of the whole, a cytoplasmic determiner, and is a condition of the material rather than any specific substance or body, but its action is important in the life of the species and active in the production of disturbances in the gametic system, when present in differing rates or reaction, in any zygote. Another peculiarity is its tendency to change rapidly in crossing within its limits to a common value of operation in F_2. As a gametic agent it is a very delicate one, easily neglected, but able to produce wide differences in the results of crossing, so that one is made to ask to what extent an agent of this character may have played the rôle of confusing us in some of the Mendelian reactions, leading to the assuming of conditions in the materials that were not really there at all. Throughout this series it is certain that when the *Ac* values are like, the conditions of the medium do not within limits have any action upon the reactions in crossing these two organisms, the resultant arrays being in all portions of the series entirely the product of the gametic constitutions present.

Rather interesting in the series is the tenacity with which each of the gametic systems retains its entirety. There are in this series no instances of the crossing of agents from one gametic system to the other, although there are plenty of opportunities for this to happen, so that the entire reaction is a most typical monohybrid one. At no point is there indication of difference in this cross in its reactions from those found and described in other monohybrid crosses. However, "monohybrid crosses" represent to me more a type of reaction than an indication or statement of the differences present in the crossed lines. In this instance there are plenty of differences that in all stages might have become dissociated from the original association and formed attachments to other systems present in the germinal material during gametogenesis. In some respects this series of experiments is different from the crossing of many domesticated races, in that in many of these there is only one species base to which are attached the agent or agents that are capable of displacement and rearrangement. In the *signaticollis* × *diversa* series, the total gametic complex acts as a unit in the operations of gametogenesis. Both are the same in action, differing only in the character and magnitude of the unit systems that are engaged in the operations.

This instance is, I believe, the simplest thus far described in the study of monohybrid crosses and represents the simplest type of operation that can at present be thought of in the intercrossing of two natural species. The only complexity that enters into the series is that due to the differing values of *Ac* and the rôle of conditions in the climatic complex in inducing changes in the gametic system through the activity of *Ac*.

The nature of fixed hybrids is suggestively indicated, as are some of the alleged "permanent blends" between species. So, too, it is strongly suggestive of the manner of production of many of the sports or mutants that occur, especially in the crossing of domesticated races, and it may well be true for the species in nature. Whether this is in any way related to the mutation behavior found by De Vries in *Oenothera* will receive attention in its proper place.

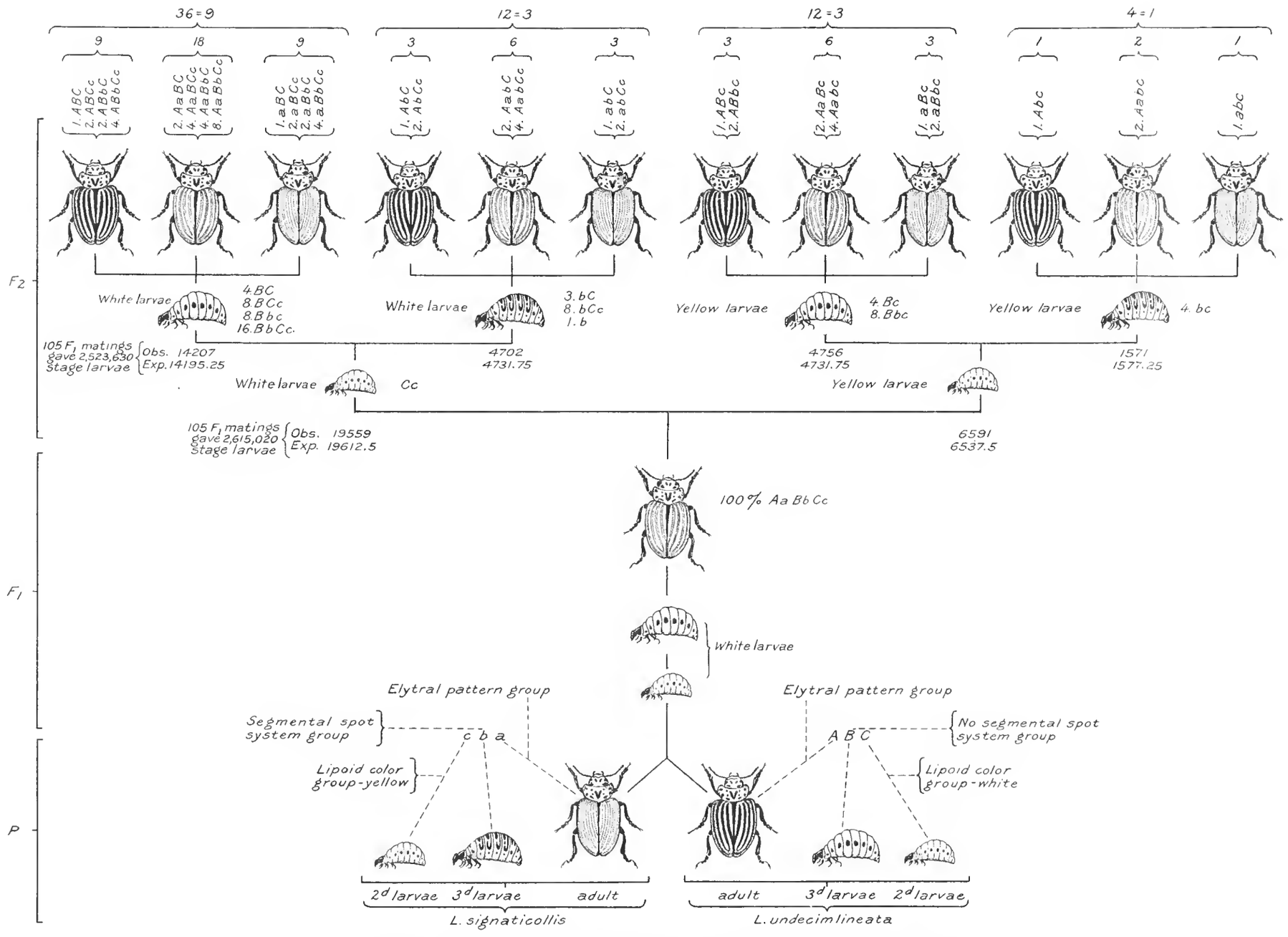
LEPTINOTARSA UNDECIMLINEATA × LEPTINOTARSA SIGNATICOLLIS.

Crossing of these two species in its simplest conditions always shows reactions of a trihybrid nature, and is, therefore, more complex in its relations and reactions than the crosses of *signaticollis* × *diversa*. These two species differ in many respects in the adult, and in the juvenile stages inhabit entirely unlike environmental complexes, with consequent differences in many of their reactions.

In the adult condition there is difference in the form index, the elytra have the same difference as in the *diversa* × *signaticollis* cross, *undecimlineata* being striped, *signaticollis* not, due to the same set of agents present in the gametes. These agents, while similar, are not in *diversa* and *undecimlineata* the same, and do not produce the same end-result; that is, the elytral complex from *diversa* is alternative to that from *undecimlineata* when the two are crossed, the differences showing in the results of the *Pu* determiner and in the character of the stripes produced by the *V* determiner.

In the juvenile stages there are differences of lipoid color and in the pattern of the second and third larval stages. In *undecimlineata* the body-color is white, with only the spiracular spots present in all of the stages; in *signaticollis* it is yellow, with only the spiracular spots present in the second and the full system of spots in the third stage. Three alternative pairs of characters are formed, no one of which is a simple one, namely, the unresolved species residue characterized by the elytral-pattern group, the larval-pattern group, and the lipoid-color group. There are others present, but they do not in this series become dissociated from the general species complex, which, as far as discovered, retains its integrity in the same manner that was present in the cross between *signaticollis* and *diversa*. These three groups of agents are "dissociated" in the ordinary operations of crossing. In addition to the agents mentioned, there are also present in this cross the differences in the *Ac* determiner found in the previous instance, only in this the average value of *Ac* in *L. undecimlineata* is, in my cultures, 64 days, same as in nature, within small range. In table 13 are given figures, from cultures of this species, in the laboratory at Chicago and in nature, showing the length of ontogeny, that is, the value of the *Ac* determiner, which is not changed in this species by introduction into the laboratory.

The two species live in quite different conditions; *undecimlineata* living in regions of high humidity and temperature, low evaporation rates, and long



SHOWS RESULTS OBTAINED IN CROSSING *L. signaticollis* AND *L. undecimlineata* WHEN THE *Ac* VALUES ARE ESSENTIALLY THE SAME AS DESCRIBED IN THE TEXT, AND CONDITIONS UNDER WHICH THE CROSSES ARE MADE ARE NEUTRAL. THE ARRAY SHOWN ON THIS PLATE IS A TYPICAL TRI-HYBRID MENDELIAN REACTION.

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seasons for development, longer even than in the habitat of *L. diversa,* in sharp contrast with the conditions in the habitat complex of *L. signaticollis.* The first results obtained in the crossing of these two species in 1904 and 1905 were most confusing.

When these two species are crossed under common conditions, with the values of *Ac* in each about 60, the uniform result is the production, regardless of the direction of the cross in F_1, of a uniform progeny that are intermediate in the adults, with the *undecimlineata* larval groups dominant when the female parent is *undecimlineata,* and with the *signaticollis* larval series dominant when *signaticollis* is the female parent. The real dominant, if such there be, is *undecimlineata,* as shown in the working out of the F_2 array. In F_2 there is produced the typical array of a trihybrid. In plate 13 is shown the results produced when these two species are crossed, when the *Ac* values are the same and the conditions in the medium neutral, and the same result is produced when the cross is made in the opposite direction, the only difference being the dominance in the juvenile stages of the female parent over the male in the juvenile character, so that the only difference between the reciprocal crosses is the dominance in F_1 of the larval complexes characteristic of the female parent.

TABLE 13.

Stock No. 722, from Coatzacoalcos, Mexico.				Stock No. 714, from San Marcos, Vera Cruz, Mexico.			
Chicago.		Nature.		Chicago.		Nature.	
Period.	Days.	Period.	Days.	Period.	Days.	Period.	Days.
Jan. 6 to Mar. 11.	64	Dec. 27 to Mar. 2.	65	June 5 to Aug. 4.	65	June 1 to July 29.	69
May 11 to July 14.	64	Dec. 29 to Mar. 1.	62	June 1 to Aug. 1.	62	Aug. 10 to Oct. 14.	65
Mar. 9 to May 6.	58	Jan. 4 to Mar. 10.	65	May 30 to July 29.	60	July 7 to Sept. 11.	66
Mar. 5 to May 1.	57	May 11 to July 12.	62	May 30 to Aug. 5.	67	July 5 to Sept. 7.	64
Mar. 20 to May 23.	64	May 8 to July 13.	66	April 4 to June 2.	59	Aug. 1 to Oct. 4.	65
May 30 to July 31.	61	Aug. 4 to Oct. 1.	58	Jan. 5 to Mar. 12.	66		
Oct. 29 to Jan. 6.	67	June 7 to Aug. 1.	55	Jan. 7 to Mar. 6.	58		
Nov. 2 to Jan. 5.	64	June 5 to July 31.	57	Aug. 5 to Oct. 10.	66		
July 25 to Sept. 29.	66	Jan. 1 to Mar. 4.	63	Aug. 2 to Oct. 1.	60		
July 20 to Sept. 27.	69	Jan. 1 to Mar. 8.	68	Aug. 2 to Sept. 28.	57		
Average.....	63.4	Average.....	62.1	Average.....	62.0	Average.....	63.8

NOTE.—The Chicago records are random samplea from the breeding stocks at Chicago, taken from F_1 to F_{10}. The records from nature are from testa of breeding in the native habitat made moatly in 1905 and 1906.

If in the crossing of these two species the conditions of the medium be those used in the normal cross of *signaticollis* and *diversa,* the dominance in F_1 is always of the female parent in larval characters, but in the adult the result is always uniformly mid-types strictly intermediate between the parents. The F_1 dominance of the female larval characters may be changed partly or completely by the conditions present in the medium, or incident upon the progeny during ontogeny, so that the aspect of the F_1 larvæ is variable, depending upon the conditions of life. Under neutral conditions F_1, while variable in the manifestation of the larval condition, is constant in the adult array and remarkably uniform in the F_2 products that come out.

While none of the groups of agents or characters that are alternative are simple, but are in instances compound associations of agents whose collective interaction is necessary to produce the character, the reaction in this cross is

that of the group collectively and not as so many individual agents, and as a result there are three groups that need be considered, namely, the mass of the gametic system, symbolized by the elytral pattern group, which for brevity may be represented by **A** for *undecimlineata,* **a** for *signaticollis,* **B** for the pattern condition in the larval stage of *undecimlineata,* **b** for the same in *signaticollis,* and **C** for the white lipoid-color group of *undecimlineata,* c for the yellow of *signaticollis.* In this cross other agents are neutral and do not act in the operation, so that the composition of *signaticollis* is **abc**, *undecimlineata* **ABC.** In all respects this series can be and is, in reality, as far as this cross is concerned, a typical trihybrid reaction, as typical and constant in its reaction as the classical case of Mendel's trihybrid peas, with this important exception. Mendel's peas had the three characters present in the same stage of the individual; in this series the three contrasting characters are not present at the same time in the ontogeny of the individuals, but at successive periods in the life of the individual, and none is in any way dependent upon those antecedent for its manifestation, so that this series is of interest as showing that the condition of trihybrid or any other is not limited to the manifestation at one stage, but that the condition governs the entire life-cycle.

In plate 13 the results shown are of the cross of these two species when the *Ac* determiners are the same in value and the conditions of the medium are uniform and neutral. The result in F_2 is that, in the larvæ in the second stage when the first contrasting characters come in, there is a sharp separation of the population into two classes, white and yellow, in the proportions of 3 white, 1 yellow, the array appearing in all instances that of a monohybrid type. In the third larval stage each of the two classes again separate, upon the basis of the pattern system present in this state, into two groups that have the general relations of 3 without pattern, 1 with pattern; but in the entire array in the third larval stage there are present four classes of larvæ: 9 white without spots, 3 white with spots, 1 yellow with spots, 3 yellow without spots, showing the "dominance" of the white over yellow and of no spots over spots, or *undecimlineata* pattern over *signaticollis.* In the adult each of the four larval groups gives three kinds of adults: extracted *signaticollis* type, *undecimlineata* type, and mid-types. These adults are always pure for the adult characters in the extracted types, but may be hybrid for one or two of the larval characters, or pure in all. The mid-types are never pure for the adult characters, but may be pure for the larval, or hybrid in 1 or 2.

That there might be no mistake, I have tested out the different products in F_2 as a routine laboratory operation, and have found that all of the expected combinations were present, giving the actual kinds of individuals that Mendel's hypothesis demands. I have found 27 different gametic conditions in the F_2 array and no more, and these were in entire agreement with Mendel's hypothesis as to their composition and their products in F_3. The combinations have been found and tested to the extent as shown in table 14.

This series has been a fortunate one, in that the condition of the larval characters is shown in the ontogeny, so that one is able with fewer matings to make an analysis of the F_2 array than if the three characters were all present in the same stage, and the fact that when *A* and *a* are present in the gamete the product is always intermediate, separates at once, in adult characters, homozygous from those that are not. With experience I learned to separate in F_2 nearly all of

the types with a high percentage of certainty, so that only a few of the matings made were other than the intended combination.

This is the typical reaction given by inbreeding F_1 mid-types with equivalent *Ac* values and standard conditions, and no exception or variation thereto has thus far been observed in F_2, and it is everywhere consistent in ratios, products, and the dominance in the larval characters of those from *undecimlineata*, regardless of whether the *undecimlineata* parent was male or female. Unlike

TABLE 14.

Appearance.			In testing the composition proved to be—	No. of pairs tested of each.
Adult.	Larvæ.			
	Color.	Pattern.		
11-lineata	Wh.	s.	ABC = ABC	9
11-lineata	Wh.	s.	ABCc = ABCc	2
11-lineata	Wh.	s.	ABbC = ABbC	1
11-lineata	Wh.	s.	ABbCc = ABbCc	14
Mid	Wh.	s.	AaBC = AaBC	2
Mid	Wh.	s.	AaBCc = AaBCc	3
Mid	Wh.	s.	AaBbC = AaBbC	2
Mid	Wh.	s.	AaBbCc = AaBbCc	7
Signaticollis	Wh.	s.	aBC = aBC	5
Signaticollis	Wh.	s.	aBCc = aBCc	2
Signaticollis	Wh.	s.	aBbC = aBbC	1
Signaticollis	Wh.	s.	aBbCc = aBbCc	2
11-lineata	Wh.	S.	AbC = AbC	3
11-lineata	Wh.	S.	AbCc = AbCc	1
Mid	Wh.	S.	AabC = AabC	5
Mid	Wh.	S.	AabCc = AabCc	2
Signaticollis	Wh.	S.	abC = abC	4
Signaticollis	Wh.	S.	abCc = abCc	1
11-lineata	Yl.	s.	ABc = ABc	2
11-lineata	Yl.	s.	ABbc = ABbc	4
Mid	Yl.	s.	AaBc = AaBc	2
Mid	Yl.	s.	AaBbc = AaBbc	1
Signaticollis	Yl.	s.	aBc = aBc	11
Signaticollis	Yl.	s.	aBbc = aBbc	4
11 lineata	Yl.	S.	Abc = Abc	5
Mid	Yl.	S.	Aabc = Aabc	2
Signaticollis	Yl.	S.	abc = abc	8
Total tested between 1907 and 1910 (pairs)				105

NOTE.—The number of pairs tested in the above table has no relation to the frequency, owing to my being able to rather accurately determine by inspection the composition and many matings made for purposes other than this test.

the cross of *diversa* × *signaticollis*, the array in F_2 shows nine different kinds of mid-types, only one class of which is comparable to the F_1 condition and occurs closely in tests and recognized counts on the average of eight times in the total frequency of combinations. All other mid-types are different in their constitution and reaction from the F_1 and the one class of F_2 mid-types. I have no doubt that this represents a typical and perfect instance of the behavior present

in trihybrids, entirely comparable with those investigated by others, and in all respects a duplicate of the classic trihybrid cross of Mendel with peas. The contrasted characters represent groups of agents associated in reaction that are transferred entire in the rearrangements of the qualities in F_2. Two points are of especial interest in this series, namely, that the characters are successive in ontogenetic sequence, a condition quite different from that present in most other trihybrid crosses examined, and the presence in the series of a meristic series of color-marks that is inherited as a unit system.

I have repeatedly made this cross in both directions, testing to F_3 or beyond, as far as space and aid permitted, and in all there has been not one indication that the behavior in any respect was other than the most orderly and typical. This statement applies only to the cross when made under conditions that are neutral to the two, and when the *Ac* determiner has the value of about Ac^{60} in both, so that the reaction represented with this complex, and conditions in the medium, is the basal reaction between the two species complexes. The same cross has given most perplexing arrays at different times, the production of which are now to some degree understood. It is important to make certain that the reaction between the gametic complexes is typical, or else to determine the agents that produce the discordant result. In table 15 I have given examples of data derived in the testing of this cross in F_2, where the *Ac* values were about 60 and the conditions in the medium were neutral.

One peculiarity present in this series is the dominance in F_1 of the larval color-characters of the female parent in the cross, unless the conditions are in one way or another inhibitory to this, a change which is easy to produce experimentally. There is no indication that the dominance is in any manner associated with sex, or any other sex-relation. The corresponding groups of determiners are introduced by the male gamete and are present in full intensity in F_2, so that there is no suggestion of the contrasted characters being absent, and the fact that the same result occurs in the reciprocal at once throws out any idea of the strength of one parent species being superior to the other. The fact that conditions in the medium were able to easily change the relations of the four groups of agents in the larval stages, as far as their manifestation in F_1 is concerned, but not in any way in the F_2 array, leads to the conclusion that the condition of apparent female dominance in the F_1 juvenile stages represents a *condition* in the mass of the female gamete, already has an initial velocity along the line from which it came, that is imparted to it by the developmental activities which produced the matured and which egg determines F_1 juvenile dominance. Therefore, the initial developmental momentum in the egg continues in the same direction these groups of agents as uppermost in visibility, until they are in one way or another, by internal or external conditions, retarded until the two sets of agents present are on a level, and then the visible dominance in the F_1 heterozygotes depends entirely upon the rôle of conditions within the organism and without it, a relation that is shown by simple experiments, so that the reciprocal F_1 juvenile arrays can, within the limits of the time and change possible with the different molts, be altered at will. This is especially true with regard to the lipoid body-colors, which can be changed about at will in the larvæ merely by the alteration of conditions. I am of the opinion that this is the cause of the conditions found in the F_1 crosses of *Fundulus*, described by New-

TABLE 15.

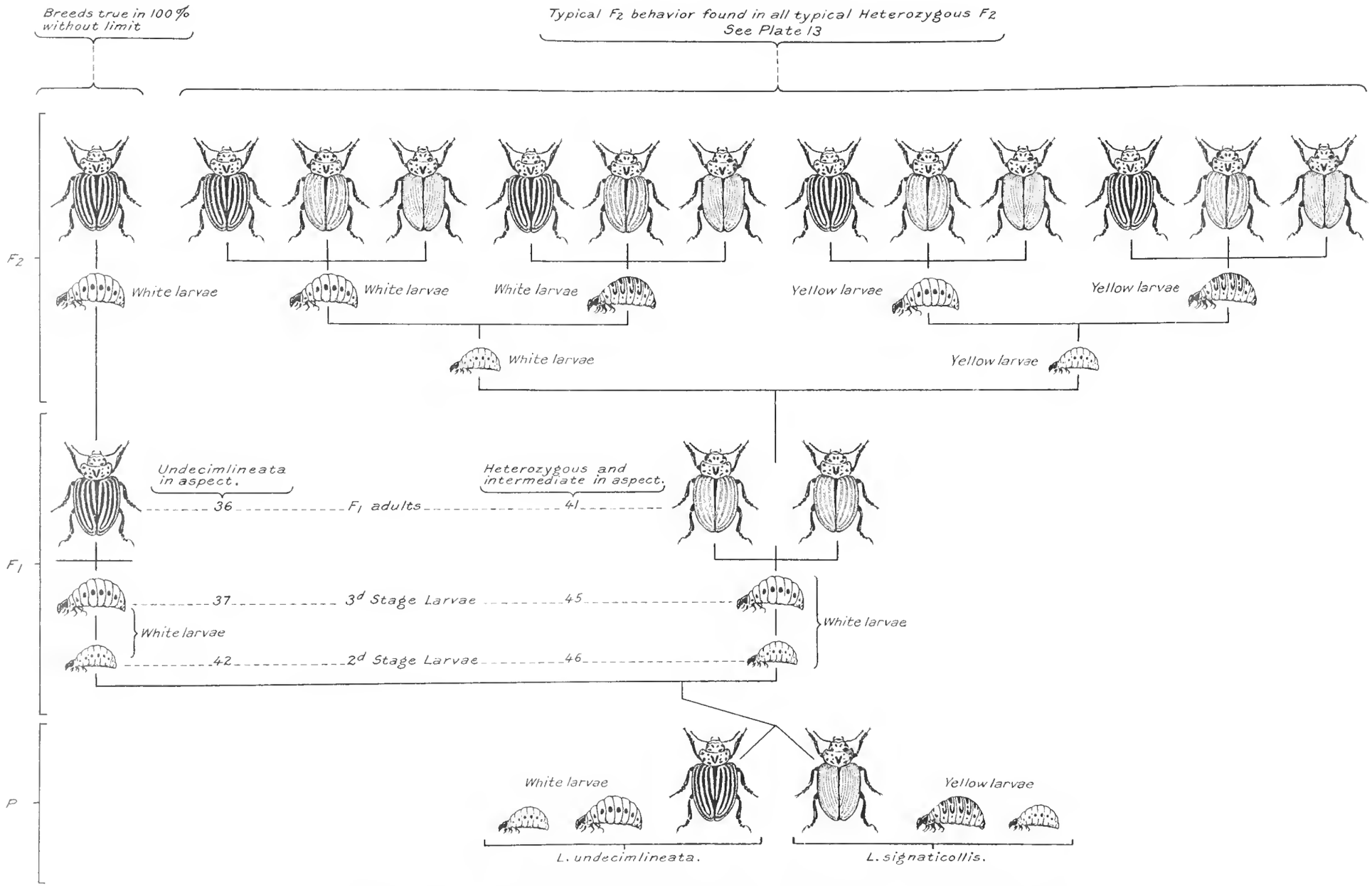
Character of cross.	Second larval array.		Third larval array.				Adult array appears in 12 groups; 3 from each of the 4, third stage larval groups.											
							wh. s. larvæ gave:			wh. S. larvæ gave:			yl. s. larvæ gave:			yl. S. larvæ gave:		
	wh.	yl.	wh. s.	wh. S.	yl. s.	yl. S.	11-lin.	Mid.	sig.	11-lln.	Mid.	sig.	11-lin.	Mid.	sig.	11-lin.	Mid.	sig.
Expsected frequency of classes in each stage as they appear.	3	1	9	3	3	1	9	18	9	3	6	3	3	6	3	1	2	1
Relative numbers of each gametic composition of members of each group of adults.							1. ABC 2. ABCc 2. ABbC 4. ABbCc	2. AaBC 4. AaBCc 4. AaBbc 8. AaBbCc	1. aBC 2. aBCc 2. aBbC 4. aBbCc	1. AbC 2. AbCc	2. AabC 4. AabCc	1. abC 2. abCc	1. ABc 2. Abbc	2. AaBc 4. AaBbc	1. aBc 2. aBc	1. Abc	2. Aabc	1. abc
Frequency of adults.	.....	.....	.....	.....	.....	.....	1	2	1	1	2	1	1	2	1	1	2	1
M. sig. × fem. 11-lin.:																		
Observed F_2	35	12	18	6	8	3	5	9	5	3	7	2	2	4	3	1	2	0
Expected F_2	35.25	11.75	19.783	6.561	6.561	2.187	4.75	9.50	4.75	3	8	3	2.25	4.50	2.25	0.75	1.50	0.75
Fem. sig. × M. 11-lin.:																		
Observed F_2	330	90	233	92	71	30	39	73	20	13	35	9	6	28	9	3	10	5
Expected F_2	322.5	107.5	239.58	79.86	79.86	26.62	33	66	33	14.25	28.50	14.25	10.75	21.50	10.75	4.5	9.0	4.5
M. sig. × fem. 11-lin.:																		
Observed F_2	348	187	248	93	116	65	43	72	32	20	46	19	7	26	12	10	27	19
Expected F_2	401.25	133.75	293.58	97.86	97.86	32.62	39.25	78.50	39.25	21.25	42.50	21.25	11.25	22.50	11.25	14	28	14
Fem. sig. × M. 11-lin.:																		
Observed F_2	301	90	107	64	50	32	26	51	24	12	24	11	10	19	8	6	14	5
Expected F_2	293.25	97.75	145.29	47.43	47.43	15.81	25.25	50.50	25.25	11.75	23.50	11.75	9.25	18.50	9.25	6.25	12.50	6.25
Total array from 40 F_1 pairs																		
Observed F_2	11,772	3,921	8,730	2,945	2,920	970	1,659	3,387	1,601	501	987	486	477	993	485	224	456	211
Expected F_2	11,769.75	3,923.25	8,755.83	2,918.61	2,918.61	972.87	1,661.75	3,323.50	1,661.75	492.6	985.0	492.5	388.75	777.50	388.75	222.5	445.0	222.5

man and others. In this case there is no indication that there is any agent or relation of the juvenile characters in the female stock that are productive of this result, and the conception advanced here seems entirely in harmony with the facts observed. I think no one will deny that the gamete, especially the female, has at the time of fertilization considerable developmental momentum, as shown by the fact that unfertilized eggs may go on developing for a time without any inciting cause, either fertilization or artificial parthenogenesis, due to the developmental momentum acquired in the growth-period, and that in this period certain directions of reaction and rates of reaction become established and continue on in the zygote, unimpeded by the cross when made under neutral conditions, so that this dominance has only a temporary existence and no genetic significance.

Out of this cross there result 8 pure breeding races that are homozygous in all respects and that continue homozygous without limit either pedigreed cultures or in mass cultures. These appear as shown in table 14, a result different from that present in the cross of *signaticollis* × *diversa*.

This series shows that in the crossing of species there is no detectable difference in the reaction from that present in crossing intraspecific conditions; in both the reaction is the product of the number of individual agents or the groups of agents that are capable of being dissociated and metathetically redistributed between the gametic systems present. I have made every effort of which I am capable to discover if this instance was not nonconformable to the results of Mendel, or that the interpretation was not correct. There have been abundant complications which will be described presently, but in all the net result has been complete confirmation of the reaction as described by Mendel in his trihybrid cross of peas. It may seem a waste to attack this instance so extensively, but the materials have served as the basis of some other investigations, and this served to test fully whether in this respect they were in accord or discord with materials used by other observers. The series has an interesting outcome in the production in F_2 of 8 arrays that are different in their life-cycle, so that there are 8 potential species which would have been described as species by taxonomists had they been found in nature and their pure breeding and constant characters been determined, and in fact such juvenile differences have lately been made the basis of separation of species of Culicidæ by different workers, no doubt with entire correctness, but is it not possible that many of them have arisen or been distributed in the series by processes of this character? The taxonomist knows nothing of the genetic sequence or constitution in his species, only the fact that the same form occurs repeatedly in the same locations, and genetic continuity is assumed, in most cases with correctness; but one is made to suspect that the idea has been too liberally applied.

The reaction that has been described may be called the *basic reaction* between these two gametic systems, and shows the extent of the dissociation of the systems that takes place under neutral conditions of reaction, and not in any way influenced by external conditions. When, however, differences in the value of the *Ac* determiner in the medium and in other agents that are present are brought into the complex, then the reactions become much complicated, producing results that, at first sight, appear entirely at variance with the principles of factorial composition and reaction, and "gloriously non-Mendelian."



SHOWS RESULTS OBTAINED WHEN A CROSS IS MADE BETWEEN *L. signaticollis* AND *L. undecimlineata* IN WHICH THE *Ac* VALUES ARE DIFFERENT BUT CONDITIONS OF THE MEDIUM ARE NORMAL.

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The most common departure from the routine condition in the crossing of these two species is shown in plate 14, in which the results are graphically given when the cross is *signaticollis* with the Ac^{40} and *undecimlineata* Ac^{62}. The conditions of the cross external to the organisms have been on the average as follows:

	Temperature (average).	Relative humidity (average).
	° F.	*Per cent.*
Day	101 ± 4	77 ± 6
Night	85 ± 3.5	97 ± 3

Wind movement, av., 440 ft. per min.; range, 425 to 455.
Evaporation (av., 52.3 c. c. per 24 hrs.):
Day (7 h. 30 m. a. m. to 5 h. 30 m. p. m.), av., 43.1 c. c.
Night (5 h. 30 m. p. m. to 7 h. 30 m. a. m.), av., 9.2 c. c.

These conditions presented evaporation-rates in excess over that found in the normal habitat of *undecimlineata* and about that present in the habitat of *signaticollis*. It has made no difference that can be detected whether the stocks came direct from nature or whether the rate of action of *Ac* had been produced in the laboratory, the result is the same.

The products of the cross are in F_1 that the larvæ divide into two groups most clearly determined in the second larval stage, yellow and white larvæ, and this division is present in the third stage, with both having the pattern-system of *undecimlineata* dominant; the larvæ are either yellow or white without spots. The numbers are highly variable but on the average are 3 white to 2.7 yellow, essentially a 1:1 ratio. The white larvæ give only adults that are *undecimlineata* in type; the yellow larvæ give only mid-types. The two classes of adults are also present in essentially equal numbers. The F_1 *undecimlineata* types when inbred "breed true" without limits, as pure-breeding races, in aspect showing only the presence of the *undecimlineata* adult and larval characters as long as the breeding is continued. Inbreeding of the mid-types derived from the yellow larvæ shows in F_2 only the normal and entirely typical array produced by the mating of typical F_1 heterozygotes between these two species, and many dozens of tests of these were made between 1906 and 1914, so that we need not concern ourselves further with this portion of the series. The *undecimlineata* types that are derived in F_1 are the portion of the experiment that is of interest.

In superficial respects in general behavior this experiment is much like the reactions shown in plate 9, in which it was seen that the two F_1 classes were different conditions of heterozygosis, and it is on the surface probably the same in this cross. There is one conspicuous difference in the apparently extracted type that comes out in F_1 in this cross that is not found in the first case, namely the *decreased* rate of reaction present in this *undecimlineata type*. Unlike the normal form which has an *Ac* value of about 60 to 64 days, or the F_1 heterozygote, which in this cross has the *Ac* value between 60 and 65 days, the F_1 *undecimlineata types* have the *Ac* value from 70 to 98 days. The actual length of time between generations is 290 to 335 days, or about a generation in 10

to 11 months, showing a considerable lengthening of the developmental period. This result is attained regardless of the direction of the cross, and uniformly so long as the conditions in the materials combined and in the medium are those given.

This cross and the results that come out are shown in table 16, in which are given the parental conditions, the F_1 results, and the breeding of *undecimlineata* types in F_2 or beyond. No purpose is served by presenting the breeding-test of the mid-types; enough has already been given to show the characteristic reactions they present.

The F_1 *undecimlineata* types have been kept in the laboratory for generations; they have been sent to Tuscon; placed in isolated locations in Mexico, and planted upon some of the Florida Keys. Nowhere have they shown any change in the length of life-cycle or in the character of the form, or any

TABLE 16.

Parents of the cross.	F_1 adult array types.		F_2 arrays and reaction rates of Ac.				F_3 arrays and reaction rates.			
	11-lin.	Mid.	Min.	Av.	Max.	No.	Min.	Av.	Max.	No.
Sig. m. Ac^{39} × 11-lin. fem. Ac^{62}	46	52								
	Pair A ...	.....	78	89	95	131	AA 73	88	97	46
							AB 70	90	98	59
	Pair B ...	.....	74	90	98	146	AC 79	89.5	93	171
							AD 83	89	92	141
							EA 77	89	93	24
	Pair C ...	.....	76	90	93	149	EB 72	90	97	146
	Pair D ...	.....	84	91	95	73	EC 87	91	95	78
	Pair E ...	.....	87	90	98	171	ED 80	91	97	92
Sig. fem. Ac^{40} × 11-lin. m. Ac^{64}	81	37								
							CA 78	90	96	46
	Pair A ...	.....	81	89	94	71	CB 79	91	97	59
	Pair B ...	.....	79	90	97	46	CC 83	89	91	193
	Pair C ...	.....	78	90	98	137	CD 71	88	93	71
	Pair D ...	.....	74	88	92	46	CE 78	90	98	44
							CF 84	90	96	139
Totals in 10 tests: Sig., av. Ac., 38.3 ..... 11-lin. av. Ac., 62.2	292	801	70	89.7	98	2,214	70	90	98	3,144

The values given are for the length of ontogeny, egg to adult, and the number observed.
Average length of ontogeny has been 90 days = A^{90}.
Average elapsed time between generations, 316 days.

other discovered change or return to the parental rates of development; nor at any point in the series did there appear any trace of the *signaticollis type* coming out of the line. In Mexico, where they were introduced into a location north of Jalapa in 1908, they persisted for at least two generations without any change that I could discover; and material taken from the location in 1910 and tested showed that the same condition was present as at the start. The type appears highly resistant to differing external conditions and is in reality well organized to persist in many different conditions of nature.

In the laboratory this type has been subjected to most varied tests in the effort to prove it to be in composition a masked heterozygote. When crossed with *signaticollis* the reaction is, when it is obtained at all, a duplicate of the reaction from which the type arose, with about equal numbers of the two types present in F_1, and the *undecimlineata type* that comes out in this test breeds true.

Thus far only one test agent has been found that serves to even partly show the composition and nature of this stable heterozygote, namely, the pure *undecimlineata*. The suspected race appears as *undecimlineata* in all respects, and the test of its heterozygous nature would be to recover from it in some manner the *signaticollis* character, either in whole or in part. When pure *undecimlineata* and the *suspected type* are crossed under the ordinary breeding conditions of the laboratory nothing results, the two breeding with perfect freedom and no separation of the types appearing; and perhaps the only reason for so persistant pursuit of the race is the constant presence in the suspected type of the trimodal condition in an otherwise perfectly uniform fraternity and series of fraternities.

By applying the same test to the fraternities that I used in the cross of *diversa* × *signaticollis* it was found that the F_2 fraternities were trimodal, and that the modes of the compound curve were essentially the same as the parental modes, with the intermediate mode that of the heterozygous type. It was further found that breeding from the modal conditions of the extreme modes at once gave constant lines so far as the form-index was concerned, and that this was in value the same as the parental modal values in pedigreed lines. Only measurements of fraternities were of any use in this analysis, as fraternities are the only homogeneous materials that we have to work upon. In figure 9 I have shown the results of a biometric analysis of the suspected race and the products of breeding in line from the modal conditions.

In many respects this would by many be considered competent proof of the heterozygous nature of the suspected line, but the final proof is to recover the suspected contaminating agents from the line. In this I have, to the present time, been only partly successful, due in the main to the lack of the requisite facilities for control and production of the necessary external conditions.

I have found that when the suspected race is crossed with a pure line of *undecimlineata* under conditions to be shortly described, there resulted in the progeny of such a cross the recovery of *signaticollis* larval characters—*i. e.*, the yellow larval color and the meristic pattern system of the third stage. These could only have come from the suspected race, as they are known not to be present in the test materials used—*i. e.*, pure *L. undecimlineata*.

When the test race is crossed with the race to be tested under the following conditions, the F_2 fraternities show *signaticollis* larval characters in widely varying proportions in about 50 per cent of the trials. The combination of conditions that I have employed are those of a desert complex, with high daily temperature and intense desiccation, and low night temperatures, with saturation, or nearly so. The average conditions were, in the tests that have been made:

TABLE 16A.

	Temperature (average).	Humidity (average).
	° C.	Per cent.
Day	42 + 2.3	34 + 6.2
Night	21 + 1.2	96 + 4.5

Wind movement by day, av., 412 ft. per min.; night, av., 10 ft. per min.
Evaporation by day, av., 94 c. c.; range 81 to 107; night, av., 2.3 c. c.

This set of conditions is rigorous and serves to stimulate or to accelerate different agents and operations in the two lines. When the cross is made between the pure and the suspected materials, F_1 shows no trace of the *signaticollis* conditions at any point, nor does it appear in F_2 unless the same set of condi-

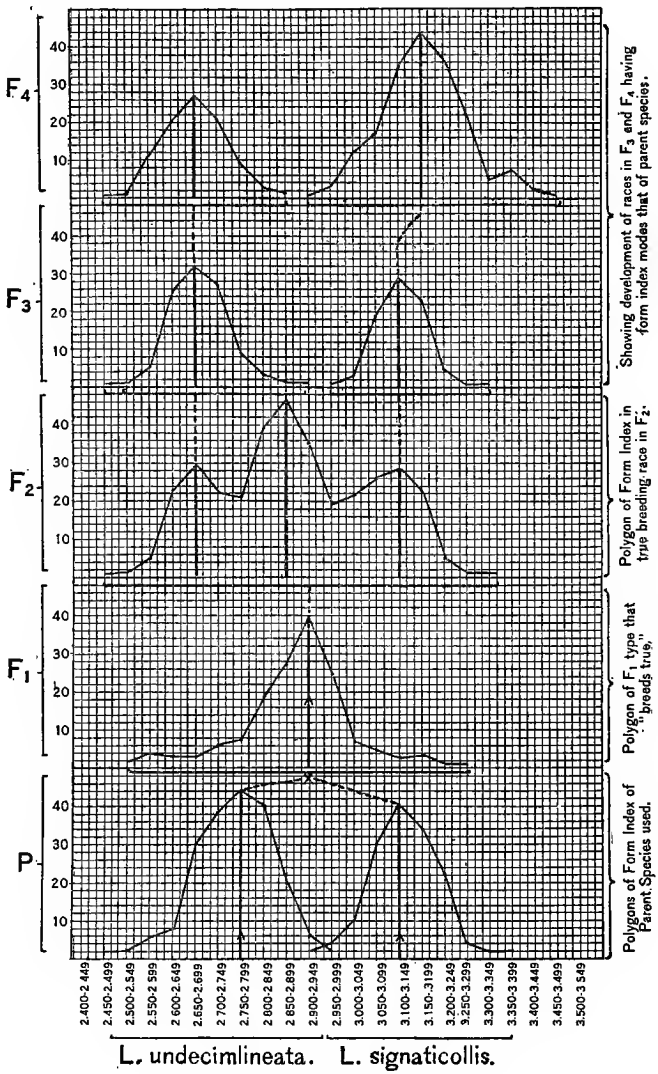


FIG. 9.

tions are applied to the breeding pairs of the two at the time of breeding; and moreover, the result is not produced unless the same complex is applied to the F_1. The mated pairs ready for breeding are introduced into the apparatus under the conditions and allowed to remain there until the eggs are deposited,

when they may be removed to the usual conditions of the laboratory for the remainder of the cycle, and then again treated as before in the mating of F_2.

The test has usually been applied to F_2 and F_3 fraternities of the suspected strains and to lines whose form-index was that of the parental *signaticollis* species. The results show that the recovery of the *signaticollis* larval characters from the different tests is by no means found in every test, nor in any regular proportions, and sometimes it is only yellow color, at others it is the meristic spot system, in others both that are recovered, showing that the operation needs further investigation.

After the recovery of the larval *signaticollis* characters from the race, they continue to breed as true and to be as accurately alternative in crossing as if they came from pure *signaticollis,* and the end-result is that from one of these tests it is possible to get certain lines with these constantly present as the larval characters in materials otherwise *undecimlineata.* No indication of the position and relations of these hidden groups of larval characters in the suspected race that I care to discuss has been determined. However, the test, in common with the trimodal condition in the F_2 fraternities in the suspected line, shows conclusively that the line is in reality heterozygous and is in all respects a suppressed heterozygous series, excepting that in this series the combination that the *signaticollis groups of agents* have made are binding and broken with great difficulty, so that only certain most precise conditions and interactions result in the recovery of the agents from the suspected complex. It would be of interest to know how long this condition would remain in the line. I have found them present in F_5 in one series and in F_6 in another, but I do not know how much longer they may remain. I do not know whether they play any other rôle in their new situation, as there is no evidence of new characters having come out in the line as a result of the contamination present.

One other suggestive test has shown that the adult elytral-pattern system is also present in the suspected race and has been recovered in the two tests that have been made of this kind. It was found that when the test was made with the same day conditions as in the first test series, but with the night conditions having a much lower temperature, as low as 10° C., with saturated atmosphere and no wind-movement and no evaporation, the number of individuals showing the larval characters increased and that a few individuals arose showing the *signaticollis* adult character.

These adult *signaticollis* characters thus recovered do not change, but are permanent in the race from that time on, and in one test produced in F_4 a pure-breeding homozygous race that was *signaticollis* in its adult characters. I have not carried this testing further, as the requisite apparatus has not been available, and an ice-box will not serve for the nocturnal low-temperature portion of the test conditions; its conditions are too unsanitary and are productive only of failure.

Collectively the results of the testing show that the *undecimlineata* type that appears in F_1 is in reality a fixed or masked heterozygote in which all of the contrasting characters are present, but, in some way not discovered, are bound in associations that are not broken excepting by certain specific and energetic treatments. There is no doubt but that in the cross between *signaticollis* × *diversa* and *signaticollis* × *undecimlineata,* when the *Ac* values are different,

there results, in conjunction with conditions of the medium, changes in the association of agents or groups of agents in the gametes, such that in a portion of the population the F_1 heterozygous condition in appearance resembles one of the parent species and continues to breed true until the gametic system is again changed and the agents freed from their position of nonvisible manifestation. In both there is the same separation of the fraternities in F_2 of the suspected race into trimodal polygons, showing that the species form-base as measured by the form-index is not affected by this firm association of the several agents in the heterozygous individuals. It is true that the series in both species is quite unusual—call it a non-Mendelian reaction if need be—but there is at no point that I have been able to demonstrate with certainty anything either in action or in result that is in any manner at variance with the principles of factorial composition and reaction, and further in the series the reactions, as far as they have been analyzed, are most clearly in entire accord with the principles of the Mendelian reaction. When it is possible to carry this further I have no doubt that it will be capable of as complete analysis as was the simple case in the crossing of *signaticollis* and *diversa*.

A feature of interest that has come out in these crosses is the slow breeding of the *undecimlineata* race that appears in F_1, giving about one generation in 10 or 11 months. As far as I know this slow breeding persists under ordinary conditions indefinitely and remains a permanent character in this race. Some indications of its origin have been obtained that are of interest. In Chapter II it is shown that in *signaticollis* the normal cycle is two generations in rapid succession, then a period of rest for about 9 months, passed either in the ground in æstivation or in part on the plants in practical inactivity. Under the conditions of the laboratory the same rhythm is maintained, but the length of ontogeny spread out and the period of quiet between successive cycles is shortened. In experiment, when the rate of reproduction is shortened by conditions in the medium, the stock always shows longer periods of rest and more uniformly enters into hibernation for this period than when the *Ac* values are larger, at about 60. On the other hand, *undecimlineata*, while it has the same rhythm of reproduction, is much less sharply delimited and the period of repose between successive cycles is shorter and to a large degree a product of the conditions of the medium.

I may later show something of the nature of this rhythm and its relations to agents within and without the organism and its general methods of inheritance. In general in these forms in pure reacting lines, in which the rhythm is homozygous, the sequence is followed with precision, of an overwintering or resting generation that emerges from the period of inactivity, especially on the advent of favorable conditions in the medium, reproduces at once, giving a summer generation that reproduces without rest, giving a second generation in the cycle, which, however, hibernates or rests in one way or another for a varying period of time. There are thus in the cycle two conditions in reproduction that follow in rhythmic manifestation—winter generation, which after resting produces the summer generation, which in turn produces a winter generation without resting, which winter generation rests from reproductive activities in *signaticollis* as much as 9 months in nature and in the laboratory strains with *Ac* values of 40 or thereabouts.

In crossing of these species it has been the uniform custom to mate in the original crosses only individuals of like condition with regard to this reproductive cycle; otherwise troublesome complications in the breeding-out of the series is sure to be encountered. Thus in practice it has been most common to mate the materials on emergence from the resting-period, as one is thus sure to get the first two generations of the series within a half year or less, and thus facilitate the operations. More rarely are matings made between the summer generations which give only F_1 before a period of rest intervenes, and only for special purposes are the two aspects of the cycle mated, when the result is complications in abundance.

These crosses between *L. signaticollis* and *L. undecimlineata* have been, as far as this portion of the investigation goes, made entirely between the stocks on emergence from hibernation, and with respect to the character of the reactions the two types of reaction described present quite different results. In the normal cross, when the *Ac* values are about the same and the conditions of the medium are neutral, the result is the regular cycle in the reproduction of the series, the second generation following at once after the production of F_1, giving F_2 within the usual period of 4 months from the initial cross, and then F_2 is followed by a period of rest in the F_3 fraternities, with a duration on the average of from 6 to 10 weeks, depending entirely upon the conditions in the medium. In the second type of result in the crossing of these two species, as shown in plate 14, the result is different. The suspected *undecimlineata race,* now known to be heterozygous, acts at once as a winter generation, commonly enters within a few days into typical hibernation, and is not to be forced into reproduction, although it may be forced from hibernation. The F_1 heterozygous types that are normal give the characteristic response by breeding at once, giving the F_2 by the end of 4 months as in the normal cross. Further breeding of the two F_1 types show that in the manifestly heterozygous series the reactions in the cycle are normal and can be carried through to at least F_6 within 12 or 13 months, while the *undecimlineata* series in this same time produces only one generation.

It appears that in this suppressed heterozygous race there are some important rearrangements produced in many of the properties present, and that there have arisen associations of the agents in the gametes, such that the arrangement present allows of only certain visible end-results. There is no doubt that the race is heterozygous, as is demonstrated by the fact that the F_2 fraternities show accurate separation of the series into a trimodal polygon, whose significance with regard to the form-index is known; that certain methods permit of the recovery from the race of the nonvisible *signaticollis* larval and adult elytral characters; and further, the race shows a complete suppression of the summer-brood production, so that the race appears in a long monobrood cycle that in general aspect is *undecimlineata,* the total complex being retained without change as far as has been carried and without regard to external conditions as far as is shown in the observations. Only special operations, under precise complexes of the medium, produce dissociation of this complex that arises so uniformly in F_1.

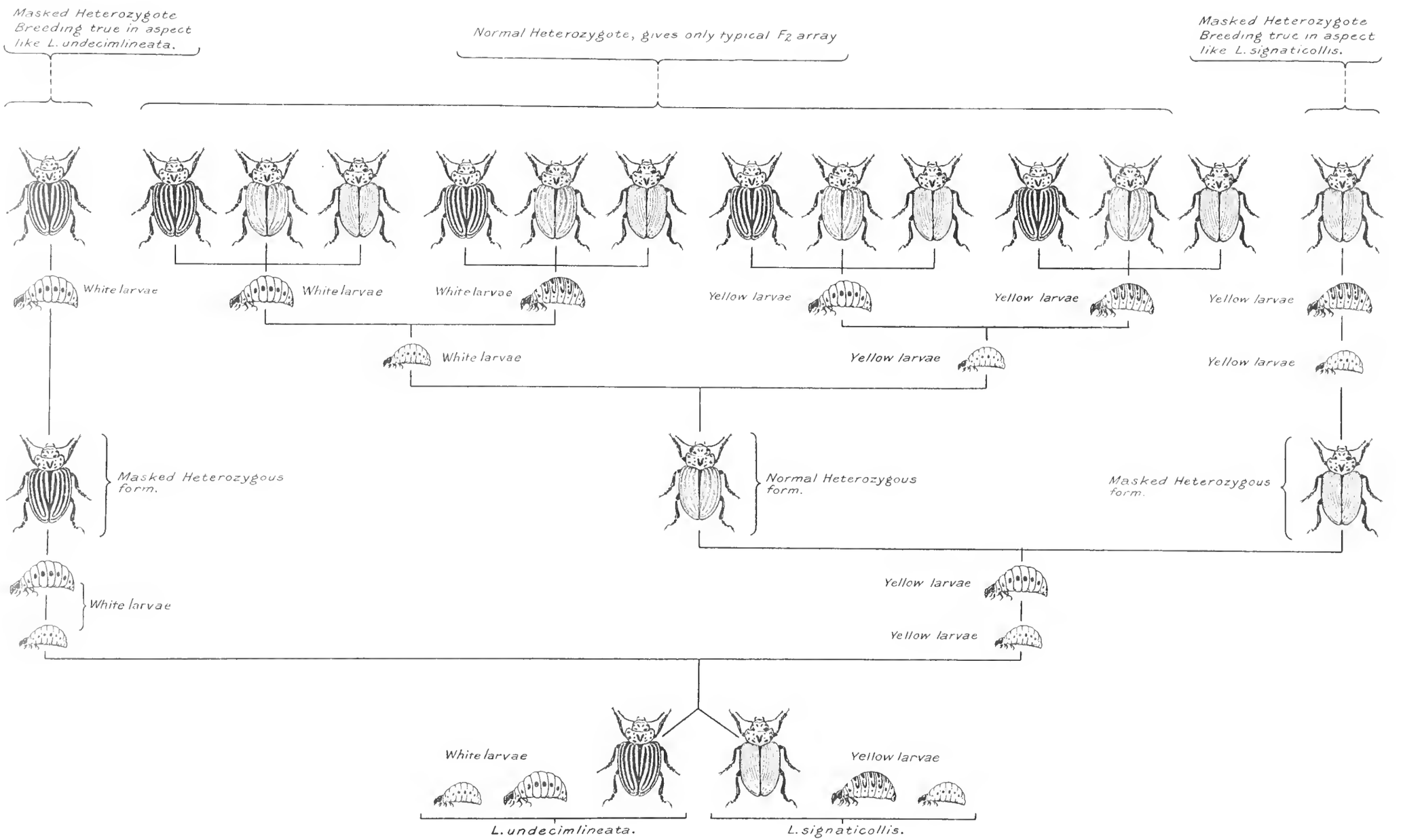
One series of this strain I carried from 1906 to the end of 1914, in which I had 10 generations after the initial one, but at the end it was the same in behavior as in composition as at the start. One point that came out in this series was of much interest. It has been shown that the F_2 fraternities are trimodal

in the form-index; that from these can be reared pure lines of the form-index; but this is possibly only by isolation and inbreeding. During the same time that these were in progress certain mass-culture lines were carried, with the result that a test of the form-index that I had made in the fourth generation showed only a monomodal curve, and the same was true in the sixth, which I also had tested. From the fourth I was able, by mating likes, to produce by F_6 much-restricted lines in the modes, so that there had taken place in the mass-breeding a mixing with the result of obscuring the real conditions present, but out of which I could isolate pure lines with respect to the form-index.

The production of this *undecimlineata* line is essentially the production of a good species in every sense. It is strong, well able to survive widely ranging conditions; the fact of its slow breeding at once isolates it from crossing back with the rest of its relatives in the production of F_2; its low power of dissociability with respect to its contained potentialities, the fact that it is produced in numbers of both sexes in the one operation, shows how in nature a new group might easily arise that would thereafter become a permanent element in the fauna of the location. Any of the usual tests of breeding would in natural materials have shown only uniformity, when in reality they were heterozygous in origin and in character, and by appropriate means the fact of this can be shown. It is not known how long the *signaticollis* groups of agents would remain in the race unseen, nor is it known what part they may be playing in the complex as it is seen, but as far as one is entitled to an opinion it seems to me that they are most probably present in some association that ties them with other agents, the totality acting as a unit system in reaction, but in which a portion of the group is relatively unimportant and may be recovered without altering the aspect of the series.

That the reproductive cycle is also in reality heterozygous is shown by the experiences in two series—one in F_3, the other in F_4 in another line entirely independent of the first, in which, after subjecting the lines to severe conditions, there came out of each a line that showed the normal rhythm with some changes. I was not able to duplicate these experiences in later tests, and, therefore, am not disposed to base more than the suggestion upon them that the experience shows that in this character of the reproductive cycle the usual double brooded condition could be recovered if we knew how to dissociate or analyze the present combinations. To all intents and purposes the line is a stable typical species, and the series shows how such might easily arise in nature. In ordinary terms this is a fixed hybrid, and while it is fixed in one sense, it is by no means irretrievably so altered, nor is the condition present in any manner at variance with the principles of factorial composition and evolution, but as far as I am able to discover are entirely a confirmation of them and an extension of their operation into a type of operation that may well have played a frequent and important rôle in the production of new specific groups in nature. But any such group, pure as it might come in the usual breeding operations, has present within it the associated agents ready, on the realization of the right conditions, within and without, for the production of new characters or races by sudden changes.

Three other main types of reaction have been found in this series, which, while in first appearance entirely at discord with any of the usual considerations in crossing, are nevertheless entirely in harmony, as far as tested with the



TO SHOW RESULTS OBTAINED IN CROSSING *L. signaticollis* AND *L. undecimlineata* WHEN *Ac* VALUES ARE DIFFERENT AND WHEN CONDITIONS OF THE MEDIUM ARE THOSE DESCRIBED IN THE TEXT.

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principles of the Mendelian reaction and the general evolutionary conceptions of the factorial theory. These in most respects are duplicates in a broad way of the differences in reaction found in the cross of *L. diversa* and *L. signaticollis,* the differences in both series being the product of the conditions of the medium in which the cross was made.

I first found, in 1907, that when *L. signaticollis* with the *Ac* value at 38 to 40 was crossed with *undecimlineata* with the usual *Ac* value of about 62 under the following complex, that the results in F_1 were further upset and productive of much confusion.

TABLE 16B.

	Temperature.	Humidity.
	°	*Per cent.*
Maximum	98	95
Minimum	59	40
Average	80.5	68

Wind-movement, 200 to 418 ft. per min.; av., about 356.
Evaporation, from 1 to 12 c. c. per hr.

This arrangement of the complex and its rapid extreme changes in the experiment, when brought to bear upon the crossing at the time of mating, maturing of the gametes, and during fertilization and early development produced a most distressing array in F_1, especially so when the behavior of other crosses of the same materials is known not to give anything but the most orderly and typical reactions. The actual result of this cross under the conditions is shown in plate 15 which shows that in F_1 there appear the three types of adults—visible *undecimlineata, mid-type,* and *signaticollis types*—in about a monohybrid ratio of 1 : 2 : 1.

The F_1 larvæ split in the second stage into two groups of about equal numbers of yellow and white, and in the third stage are still, in equal numbers, yellow and white, both without the meristic-spot series, so that the *L. undecimlineata* was the dominant one in this respect. The white larvæ always gave types that were *undecimlineata,* that in all respects are duplicates of the F_1 appearing *undecimlineata* types of other series, slow breeding, with difficulty dissociated, in fact masked heterozygotes. The yellow larvæ gave two types of adults, on the average of two *mid-types* to one *signaticollis type.* The *mid-type* gave only the normal reaction, and were in no observed respect different from the other F_1 heterozygotes that have been seen and tested.

The *signaticollis* type that appeared in F_1, however, was pure-breeding, at least to the extent that they came true to type for an undetermined number of generations. They were a fixed type arising in F_1 that bred true to type and had all of the characters of the *signaticollis* normal type. They bred at once, had about the same length and type of rhythm in reproduction as the mid-types, but were true to the F_1 condition in subsequent generations. These were another type of heterozygote, as could be shown quite readily by applying the test reaction used in the cross of *diversa* and *signaticollis,* which showed the recovery in F_2 following the test of the *undecimlineata* type. Biometric tests of the pure breed F_2 also showed in two fraternities that I had tested the typical

trimodal condition. The numerical results attained in this cross are shown in table 17, which shows a close approximation of the array in the F_1 to the monohybrid ratio of 1 : 2 : 1.

TABLE 17.

Parent.	F_1 larvæ, third stage.		F_1 adult array.		
	White.	Yellow.	White larvæ, 11-lin.	Yellow larvæ.	
				Mid.	Sig.
Sig. m. A^{38} × 11-lin. fem. A^{64}	121	246	41	89	33
Sig. fem. A^{42} × 11-lin. m. A^{62}	54	96	21	44	11
Sig. fem. A^{39} × 11-lin. m. A^{63}	28	21	4	7	5
Sig. m. A^{41} × 11-lin. fem. A^{62}	11	36	2	31	1
Sig. fem. A^{37} × 11-lin. m. A^{66}	39	11	27	4	0*
Sig. fem. A^{40} × 11-lin. m. A^{60}	11	84	4	46	27
Sig. fem. A^{43} × 11-lin. m. A^{60}	12	23	11	11	10
Sig. m. A^{40} × 11-lin. fem. A^{61}	71	14	63	2	2
Sig. m. A^{38} × 11-lin. fem. A^{63}	93	201	74	136	62
Totals	440	732	247	370	152

* 1 dead.

Continued breeding and testing of the F_1 heterozygous *signaticollis* strain gave many indications of the reactions and associations that had been formed in the production of it.

This line, when treated with the test reaction used in the cross of *diversa* × *signaticollis*, showed in F_2, following the test, the uniform appearance of the type in which the *V* determiner in the elytral-pattern complex had been brought into relation with the *Elp* determiner, the two producing a general color distribution over the elytron. Also, there were produced types in which the *V* dedeterminer manifestly had relations, productive of the strain where the pigment was linked with the *Pu* determiner. These two conditions have been tested out and their composition determined by the test reaction used in the *signaticollis* × *diversa* cross, and from each of them the *undecimlineata* type can be recovered by the same methods in the same ratios that were found in the first example. In fact, this *signaticollis* race that comes out in F_1 of the cross is in its adult characters the duplicate of that found in *signaticollis* × *diversa* in F_1, in so far as reactions and principles are concerned.

In the larval characters the stock breeds true to the yellow with the meristic-spot system in the line as it arises, but it can be broken and is broken up by the test reaction, giving resolution into the component character agents that went into it, showing in all respects its complete heterozygous character.

The point of essential interest in this cross under the stated conditions is the production of three sorts of heterozygotes, differing in their aspect, not in composition, but in arrangement of the agents in the gametes and in the reactions of them in development. Two are of especial interest in that they breed true, are stable associations in reactions and in gametic associations, which can, however, be broken up into the orginial agent groups of which they are composed. The F_1 production of the three types, while in the summation is an approxima-

tion to the ratio of 1 : 2 : 1, in individual experiments shows wide ranges therefrom, and these are in close correlation with the differences present in the duration of the ranging conditions used in the medium. The range shows oscillations towards both of the extreme or pure-breeding conditions, and in nature the same cross might well be productive of different results and sequences of consequences in the local fauna as the product of the conditions surrounding the initial cross.

The last complication in this series of experiments that need be mentioned here is the crossing of these two species, under conditions of high temperature and moisture with little evaporation and air-movement. The following complex of conditions with these materials has given the result that the progeny in F_1 are all alike and of the *undecimlineata type,* breed true, and appear as if the other parent had gone from sight. This result I have thus far obtained only when the female parent was *undecimlineata,* in 12 trials out of 19, giving in F_1 217 adults. The converse of this has not been produced when *signaticollis* is the female parent. These crosses have been tested in three series, and by the tests that were applied to the other F_1 pure-breeding types are shown to be in all respects heterozygous and of the same composition and apparent ease of dissociation; likewise they have the same slow-breeding reactions that are present in the other strains of the same origin. Aside from this aspect these last experiments are without much interest.

The intercrossing of these two species under different conditions shows the production in experiment, and the same no doubt is true in nature of 10 pure-breeding strains, or races that are different, so that in nature they might easily be described as species. By pure-breeding I mean only the usual application of the term, the production of progeny that are the duplicate of the parents, a term and description that are not of much significance at present. The 10 pure-breeding lines show two groups: (1) a group of 8 that are *homozygous in action and in constitution,* and (2) a group of two that are *homozygous in action, but heterozygous in constitution.* In appearance these are as follows:

(a) Homozygous in constitution and in action, always derived as F_2 extractives.

L. signaticollis (*a*) *signaticollis* in all respects, with yellow-spotted larvæ.

L. signaticollis (*b*) *signaticollis* with white larvæ, spotted with black in third stage.

L. signaticollis (*c*) *signaticollis* with yellow larvæ, not spotted in the third stage.

L. signaticollis (*d*) *signaticollis* with white larvæ, not spotted in the third stage.

L. undecimlineata (*a*) *undecimlineata* with white larvæ not spotted.

L. undecimlineata (*b*) *undecimlineata* with larvæ white spotted with black.

L. undecimlineata (*c*) *undecimlineata* with larvæ yellow spotted with black.

L. undecimlineata (*d*) *undecimlineata* with larvæ yellow spotted with black.

(b) Homozygous in action, heterozygous in constitution and always derived in F_1.

L. undecimlineata (*e*) *undecimlineata* with white larvæ not spotted.

L. signaticollis (*e*) *signaticollis* with yellow larvæ spotted.

These two groups are alike in reaction under ordinary conditions and unlike in constitution, actually and in principle, but ordinarily would be rated as homozygous, and so used in the further crossing of them. The first group offers nothing of interest or difficulty and is the product of the usual metathesis of groups of agents that takes place in crosses of the type where there are three agents or groups of agents capable of dissociation and rearrangement. The latter group would in usual operations be interpreted by the assumption of some agent in one parent not present in the other, one being heterozygous, the other homozygous, therefore with the result of the production in F_1 of the extracted type. That this is not the case in these experiments is shown by the fact that from all of these in F_1 races it has been possible in varying degrees to recover from them the other characters, which would not be possible were they true homozygous extractives. There are thus available 10 homozygous-acting kinds of substance, 8 of which are homogeneous in composition and action, 2 in action only, and in addition there are the diverse kinds of heterozygous combinations.

LEPTINOTARSA UNDECIMLINEATA × LEPTINOTARSA DIVERSA.

Crossing of these two species is simple, and also serves to show that the reactions are normal metatheses of the character groups involved. Between the two there are the larval character groups of lipoid color, yellow or white, and the spot system or its absence, while in the adult the elytral-pattern system of agents, while not precisely alike, is with difficulty separated, so that it is not so clearly a diagnostic character in the adult as they were in the former crosses. However, the antennæ in their coloration serve as a certain mark of recognition for separating the two adult conditions. This is not in this type of cross dissociated from the general species complex, as far as known, always appearing in correlation with the form-index of the proper species. The basal joints in *L. undecimlineata* are light in color, the distal are black; and in *L. diversa* the entire antennæ are black. This in common with the index serves well to separate the adult conditions. In both the *Ac* determiner values are alike, so that no derangement of the crossing reaction occurs, and the conditions employed, unless in extreme ranges, do not change the array. Reciprocal crosses show identity of F_1 results, giving yellow larvæ with the spot system minutely present or not visible, so variable in this manifestation that precise statement is possible only for individual instances, due to the fact that slight variations in the conditions of the medium produce oscillations of the manifestation of this character. The adults are also an intermediate in all of the characters, as antennæ, index, pattern, and so on. The production of F_2 from these shows separation of the larvæ in the second stage into white and yellow; into an array in the third stage that consists of 9 white without spots, 3 white with spots, 3 yellow without spots, 1 yellow with spots, and each of these give three classes of adults that appear as one, but that can be separated on the basis of the antennæ, the form-index, and to some extent by the elytral pattern. There is no indication that the reaction is in this instance anything but of the most orderly trihybrid type in all respects.

With this combination I have not attempted many crosses when the *Ac* determiner had divergent values experimentally produced, because of the technical difficulty that the separations of the adult types were slow and must be

done by either biometric testing, which is time-consuming and expensive for extensive operations, or by minute examination of antennal characters or the elytral punctations. Further, there were not found results or reactions differing in principles. The crossing of the two has shown only the fact that nothing present appeared or was brought out that was hidden, and might have been productive of the complications found in the previous series.

DISCUSSION OF RESULTS DERIVED IN CROSSING THE SPECIES L. DIVERSA, L. SIGNATICOLLIS, AND L. UNDECIMLINEATA.

I have presented at some length and in detail the crossing of these species, and out of these experiments, which were begun in 1904 and are by no means terminated, certain positive advances have, I believe, been obtained, in addition to many aids in the analysis of the composition of the constitution of the germinal material. Begun at a time when the furor of enthusiasm resulting from the recovery of Mendel's work was at its height, I purposely took a most uncompromising position, and in every possible manner made effort in this and other materials that I was using to break down the principles. The reason for this was that I believed that if the principles were true, and not another variety of Weismannism, no amount of adverse investigation would alter the findings and might open the way for extensions of the principles in diverse directions. I have no hesitation in saying that I made every effort of which I was capable, aside from biometric messing-up of heterogeneous things and assuming homogeneous arrays, and disregard for the accuracy of technical operation, to break down in practice the Mendelian reaction in these materials. To this end I made especial efforts after it became increasingly certain that the reactions were in accord with the principles of reaction described by Mendel.

In all of this I have most positively failed, and not one certain instance have I been able to find that will stand the tests that it has had to undergo in my examination of it. Mendelian enthusiasts will say that the effort was a waste of time and energy, and had far better been employed in adding to the array of cases. I have no interest in added cases, only in principles, their validity and extension. It was further necessary to test fully the rôle in these forms of this reaction, in which other operations directed at the problems of evolution were being carried on.

Current neo-Mendelian conception and terminology characterized the operations in the gametes productive of the differences and arrays that result as due to the " segregation " of factors and determiners, that all too often are conceived of as " carriers of characters," or as " specific nuclear substances," the characters being isolated units, compared to blocks in a wall, the entire terminology and conceptions in general being modified Weismannism. The experiences with these crosses of natural species shows that there is no general shifting of characters or units of any sort, either as agents or as conditioning entities. In all it is seen clearly that there is a gametic system characteristic of each species, and two systems when combined in crossing are not able to form permanent combinations, but separate at once in F_2, and this regardless of the visible differentiation. For proof of this, the uniform experience with the fixed heterozygous races, which were in appearance alike and uniform, were shown by accurate measurement to be not like, but different, and to produce groups whose

modes were the modes of the parent forms from which pure lines could be grown, pure for the parent form-index, showing that the two parental basic gametic systems did not intermingle; but while they might react in common in the heterozygote, as they must or perish, at the first opportunity they separated, each into gametes that were pure for the species gametic system in question.

In the cross between *diversa* and *signaticollis* this is all that took place, the two systems maintaining their integrity throughout the series, and no indication of metathesis being found in the series. This to me seems the simplest reaction that one can find in the crossing of two forms of organisms and is the simple interaction of two systems that are sufficiently like in substance, structure, and reactions so that in combination they are able to react as one, producing the compromise end-results of the heterozygote, which in most respects are intermediate between the two parent forms, but not able to form any permanent association as far as experience has shown, and between which no interchanges of agents or groups of agents take place in the interactions that go on during ontogeny and in the gametogenesis of F_1.

Quite different are the reactions and results in the series shown in the crossing of *signaticollis* and *undecimlineata,* in which there is the same retention of the integrity of the gametic system; but in the reactions incident to gametogenesis in F_1 certain interchanges of groups of agents occur. The array has the appearance of a trihybrid, owing to the fact that the three pairs of masses are present and interacting: (1) The non-dissociated gametic mass, characterized by form-index, elytral pattern, etc.; (2) larval pattern; and (3) larval color. These present the array of three independently interacting characters. In reality the elytral system is not in any of these dissociated from the basal gametic complex, and is the indicator of its presence, a fact that can and has been tested by breeding and by biometric measurements in the diverse lines with full confirmation in all respects. That which happens in this instance is the separation of the basal systems in gametogenesis into two great groups, distinguished by the elytral-pattern sign, and then the interchange of the two other groups of agents in metathesis, such as would in all reactions take place between unlike complex substances when in mixtures and under conditions that permit of interactions and interchanges between them.

With considerable possibility of truth one can symbolize these substances and reactions in one's imagination as two collodial mixtures, each a highly organized system in itself, with structures and reactions such that they may for a time interact in common to produce the soma of F_1, but are not able to form permanent combinations. We think of this simple relation in terms of a single pair of reacting groups of agents, and all of the evidence from F_2 separations, measurements, and breeding of extracted lines gives support to the symbolic conception of the two gametic systems retaining their identity and integrity throughout the operation. There is no evidence that the gametic systems in this cross are impure or contaminated in any way by the passage through the cross, nor are extracted stocks and parent species different in any point that has been detected.

The cross of *signaticollis* × *undecimlineata* shows in essence the same general operation as in the simpler cross, with the exception that in gametogenesis the two gametic systems which retain their individuality in the divisions of the germinal materials into the two main types of the parental stocks, with respect

to the larval color and the meristic pattern system of the larvæ, permit of interchange or metathesis of these groups between the separating systems of the groups of agents that are productive of the characters in question, and these interchanges seem to be equal in both directions. How this is produced or what it is that passes between the two systems is unknown, but it is clear that something does pass from one to the other system; that there is true metathetic change between the two interaction masses that represent the germinal substance derived from each parent and which are separating from the combination of them into masses pure for the substance contained therein, with the exception of such additions or removals as may have been produced by the metathesis resulting from the interaction of the two gametic masses. On the surface it appears much like some type of mass-reaction, accompanied by interchange between the two interacting masses. These interspecific crosses show that the mass of the gametic system, acting as a whole, as an indivisible unit of reaction in one combination, in another allows of interchanges in parts of its system, resulting in new pure breeding combinations.

These results raise the question whether the changes found are the extent of possible change, or whether there is a specific residue which may or may not be incapable of dissociation and recombination. Bateson and others have in some measure pictured the conditions as the combination of a basal portion upon which is placed or attached the agents capable of being changed in position and recombined. This basal portion is simply the unknown unanalyzed residue of the gametic complex, and there is no reason to expect a specific indivisible base upon which to build, and if the principle of factorial constitution and operation is true in part it seems most probable that the same condition holds throughout; at least that is the condition that one finds in unorganized substance, and is, no doubt, true for all substance.

CHAPTER V.

REACTIONS AND PRODUCTS IN INTERSPECIFIC CROSSES.

In Chapter IV, data and the analysis of the crossing of species from nature were given, showing interesting possibilities for the production of irregularities in the laboratory, as well as potentialities for the production of natural groups capable of continuation and independent existence in nature.

Clear indication was presented that the gametic complexes behaved as units in the production of the gametes in F_2 and of the purity of the types found in F_2 as determined by genetic testing and biometric measurements. Between these some interchange of factorial groups was observed, as well as the formation of combinations that were permanent and stable. These latter were, however, capable of dissociation, showing the manner of their production and the nature of their composition. In this chapter is presented further data derived from the crossing of species from nature.

LEPTINOTARSA DIVERSA × LEPTINOTARSA DECEMLINEATA.

The cross between these two species is difficult to make, and out of many trials I have thus far obtained two series that gave progeny that would breed on into F_2 and beyond. Many of the trials have given juvenile stages, fewer adults, only two lines that were able to breed through several generations.

The species differ in almost all of their attributes, not only in the color and pattern characters presented in all of the stages of each form, but also in the form-index, the rates of ontogentic development, in the food that each is able to utilize, as well as in many other characters. Perhaps the chief difference that is of interest in this cross is the specificity of the food-relation presented and which enters into the results of the cross. *L. decemlineata* normally feeds upon *Solanum rostratum, S. tuberosum, S. nigrum,* etc., but will eat some few of the woody types, but none of them with success. *L. diversa* will not eat the first-named types; as far as experience goes it feeds only upon the woody types that are its food in nature (*S. hegerii, S. chrisotrichum, S. lanceolatum,* etc.). So specific is this relation that *L. diversa* will starve before it will eat potato or *S. rostratum,* while *L. decemlineata* will eat the food of *L. diversa* when forced, but never thrives thereon, and in all instances perishes by continued use of the *L. diversa* food.

In the crosses between these two species I have used only materials of *L. decemlineata* that were pedigreed and came from my oldest line, and of *L. diversa* also only pedigreed stock. Between these two species I have made over 200 trials, always in reciprocal pairs, and thus far only two matings of *L. decemlineata* female × male *L. diversa* have given lines capable of any analysis, and these were both in all respects precisely alike. The crosses were made under the conditions of the breeding-quarters, to which both species had been fully

accustomed for generations, and their progeny and testing thereof was all done under the same conditions, so that the conditions of the medium play little or no part in the history of this combination.

On July 18, 1907, a pedigreed *L. decemlineata* (F_{12} female) was mated with a pedigreed F_9 male *L. diversa*. Both were young, freshly emerged, and typical of their respective lines. These gave progeny that in the larval stages were in all respects precisely that of the *L. decemlineata* type; from these there emerged on September 18, 11 male and 8 female adults that were all of the *decemlineata* type. On July 12, 1908, a precisely similar mating was made that gave progeny from which were obtained on September 16, 22 males and 25 females. In this series all the juvenile stages in all respects were indistinguishable from *decemlineata*, and the adults were also fully of the female parent type. In both of the series they were the only pairs out of 20 in the July 1907 mating, and out of 32 in the July 1908 series that gave progeny from which adults were obtained.

In making these crosses it is necessary to have in the cage two different food-plants—that of each parent; otherwise the chances are entirely against obtaining any reaction from the mating. Under these conditions the female *decemlineata* will deposit the eggs irregularly on both of the plants, with an average larger number on the normal food than on the other. These eggs, when they hatch, give larvæ that do not feed on the succulent type with any degree of success, and in those larvæ that are on the potato the mortality is, if left thereon, always 100 per cent. If on the woody type, or transferred thereto, they thrive well and in the two series there were 75 larvæ in the 1907 set and 82 in the 1908 set, all healthy, well nourished, and the progeny that came from them also were fine, large specimens, and like the larvæ would only feed upon the woody type of food-plant. In the length of ontogeny F_1 showed the rate characteristic of the *diversa* parent, which was about 60 days, whereas the same value in the *decemlineata* parent in the strain used was 38 to 40 days.

These F_1 hybrids were clearly composites, showing in gross bodily qualities entire dominance of the female parent's characteristics; in rates of growth, and in food preferences the strict limitations of the male parent. In other attributes difficult of statement, these individuals showed the type of reaction now of the male, at other times of the female parent, but in all respects uniformly so, the entire population composed of the two fraternities being strikingly uniform. In both cultures the F_1 fraternity entered into hibernation by the end of September, remaining until the following March, when they emerged, and were tried first upon potato, which was not touched, then put upon *Solanum chrisotrichum*, which was at once eaten, and by April 1 reproductive activities had begun. In April 1908, I mated 7 pairs of the first series that arose in 1907, and these bred at once, all 7 pairs giving progeny, which emerged between June 5 and 15, having an average ontogeny of 61 days. The record from the pairs is shown in table 18.

The larvæ were without exception all pure *decemlineata* type, like the female-parent stock, and the adults were all like the F_1 fraternity, with no trace of separation into types. Biometric tests, made of fraternities from matings A and D, gave no indication of multimodal conditions, the findings in the measurement of the form-index showing a monomodal polygon that was in all respects precisely that of the pure *decemlineata* stock in position and distribution of frequencies.

From pairs A and D matings were made and began breeding early in July, giving adult progeny that had all emerged by September 12, and then hibernated. These F_3 fraternities gave 416 larvæ and 318 adults in all respects like the populations in F_1 and F_2, and one line was continued to F_6 without any indication of change either in the characters or reactions of the line.

Efforts were made to cross this line with *L. diversa* and with *L. signaticollis* as well as *L. undecimlineata* without success, but it crossed feebly with *L. decemlineata*. This cross, made in the effort to break up the combination, showed not the breaking up of the combination but its dominance in F_1 and the separation in F_2 of pure *decemlineata*, and the pure combination, which could be easily distinguished and tested by their rate of development and the limitation of food. In no test did I succeed in breaking up the combination that had been formed by the cross.

TABLE 18.

Pair.	Larvæ.	Adults.	
		Males.	Females.
A	42	8	16
B	92	31	26
C	126	56	27
D	154	61	49
E	27	12	11
F	89	33	31
G	177	68	54
Total	707	269	244
Total adults..		513	

Both of the series that have been observed behaved alike, were in all discovered respects the same, and neither yielded to any of the efforts to disrupt the combination. That it was a combination there is no doubt. The virginity of the females was certain, the fact of the cross equally sure and proven by the presence in the combination of characteristics that could only have come, under the conditions of experiment, from the male line, and in all respects the product of the cross was distinctly a combination of the lines, even though the form and usual taxonomic characters were of the female type entirely. The length of ontogeny, the food limitation, and the many peculiarities of action that came in with the male line showed fully the existence of a combination of the parental lines into a complex that did not dissociate in the production of F_2 or subsequent generations. A new and stable type had arisen by this combination that was stable from the start, existed as a group from the start, that with its slow progression in ontogeny and limitation in food would have, in nature, effectually isolated it from possible intermingling with the female-parent species, while it was unable to cross with the male-parent stock.

It is not difficult to imagine the advent of a female *L. decemlineata* into the habitat of *L. diversa*, and perchance if the crossing took place there would be produced in the location a new species, if its origin were not known, that would feed with *diversa* upon the same food plant, but not cross with it, at least with any ease; that would be by food-relations, growth-rates, and other agents

isolated from crossing with *decemlineata,* or if it did cross would retain the purity of its type; so that the conditions are not unfavorable for the origin of a group in nature, the "origin of a species."

I made the effort to put this hypothetical case to a test by the introduction of *L. decemlineata* females into some of the habitats of *L. diversa.* I have not thus far been successful, owing to practical difficulties in the control of the test and the fact that *L. decemlineata* does not thrive in the habitats of *L. diversa* in nature. In 1908 introductions were made at five places in the forested valleys to the north of Cocomatepec, Vera Cruz, Mexico, where the food and *L. diversa* were found in isolated locations. At these I saw the copulation of the *L. diversa* males with the *L. decemlineata* females that were fresh from my laboratory stock. I could not remain to observe the outcome, but when the colonies were visited in August 1908 and again in 1909 no trace of the introduced stocks was found. I saw the locations again in 1910, but found no indication of the success of the test. I hope at some future time to repeat on larger scale and with better means this or similar lines of experimentation.

The series of experiences with this cross and its products is highly suggestive. The crossing of widely divergent types in the genus is of interest; one a temperate savannah, the other a tropical montane rain-forest type with different food-relations, the two differing in nearly all characters throughout; and only in the general type of structure and in the large relations of parts and in general pattern plans do the species compare, as is shown by the comparison of the figures given of these types in Chapter II. The finding that less than 1 per cent of the matings give progeny that are able to mature and continue the line, and then only from one side of the cross, shows plainly that the problems presented in this combination are by no means solved. The lack of separation of the F_2 population into types of any sort shows that there is no gametic segregation or production of gametes of unlike kinds present. All indications show that the gametes are like and permanent as far as experience goes in their combination of the characters. The character of the strains that have arisen indicates that the two parent types have each given to the new complex certain of their determiner groups and that others have been lost or else are present in inactive state or serving other purposes.

All examinations and tests that have been made show that the type in body-index is that of the *decemlineata* parent, and with this are the many characteristics of structure and color-pattern, all those of the *decemlineata* type in pure form. This, perhaps, indicates that the basis of the new combination has been the gametic system of the female from which have been lost certain groups of agents that have been replaced by other gametic agents from the male line. As far as I have been able to discover, the limitation of food is complete, and associated with this are a considerable series of reactions that involve the entire body. The diverse catalitic agents that are engaged in the metabolic activities and many sensory reactions are also distinctly present in the new type that came from the male. The rate of development and many developmental details are also those of the male line, so that the type is most complex.

It is idle to speculate upon what has taken place in this combination, and only further investigation can help in the solution thereof. It is clearly not like any of the fixed heterozygous conditions thus far encountered. These have all

yielded to test and have been capable of some dissociation, showing the presence of the lost characters in the erratic type. In this no indication of dissociation or disintegration of any sort has been produced, and the type is as stable and fixed in all tests thus far made as are any of the types direct from nature which we call species. I can only suspend judgment as to its production. It would be of interest to know the reason for the limitation of the production of these lines to one side of the cross and its realization in less than 1 per cent of the total crosses made. These are problems for the future, and as time and opportunity permit I hope to be able to penetrate farther into the mechanism and nature of this method of the production of stable, physiologically isolated groups. This represents perhaps the most complex type of interspecific cross, with complete permanent combination and production of new groups, that of *diversa* × *signaticollis* the simplest, with no dissociation and the non-contamination of the gametic system by their entering and passing through the F_1 hybrid combination. Between these two extremes are all sorts of intermediate conditions, some of which have been given in the preceding chapter, and others are to be presented in the remainder of this.

The point of most interest shown in the series is the reaction of the nutritive activities as a unit group being retained in one totality in the combination in dominant positions, so far as the feeding and foods that are capable of use are concerned. Interesting new relations must, however, have been established in the new combination, so that the products of this new nutritive arrangement and limitation may be used by the agents productive of the characteristics of the female line. The adjustments that have been made in the type to bring this about would be of much interest if known, and might give important information regarding the inheritance of metabolic relations and capacities, as well as of the gametic agents present that are responsible therefor. It is certain that the agents in the gamete, productive of restricted nutritive relations, are as specific and capable of dissociation and metathesis and of entering into new associations as are any of the other gametic agents experimented with. Further, they have the ability to produce in the new position some of the same end-results that were shown in the original association, and this in many respects is suggestive of the transfer only of determining agents, rather than the transfer of the total nutritive complex. At any rate, it is certain that agents of the most fundamental nature are able to act as units in the interchange, and recombinations have taken place in the production of this race. Other experiments in which the food-relations are subject to analysis have shown the presence in the gamete of a general basic growth and metabolism factor, with which are associated several determining agents or determiners that may be dissociated and replaced in the combination. In this series the probability is that the food determining agent, determiner F_0 from *diversa,* has combined with the chromatic receptor of the female parent, CMR^1, the combination being productive of the limitation as to food. What became of the determiner F_0 from the female line remains to be discovered.

The importance of reactions of the kind here described in evolution in nature, I believe, merits attention. By this method of reaction there arises at once a precise, isolated group with capacities and characters derived from its progenitors fitting it to be successful from the start as an element in the fauna of

the location. The agony of origin, of initial stages, and struggle for place is not seen; the type is produced or not as the result of a specific reaction, and at the close of the reaction is completed as far as that reaction is concerned. In setting, operation, and result the series is purely mechanistic and devoid of the uncertainties of time-elements and time for fixation needed to produce stability of type.

In the instance here presented a precise reaction is produced that in about 1 per cent of observed instances results in a specific stable combination—that from the start is physiologically isolated, and in all observed respects the equivalent of a natural species and fully able to survive in nature. It arose only in experiment. Thus far I have not been able to obtain it in experiment in nature, but my attempts are not thorough nor extensive in this effort. I am convinced, however, that the type of reaction seen shows clearly that the realization of an operation of this sort in nature would at once produce a combination which, if found in any habitat and of unknown origin, would pose in faunistic lists as a species or other taxonomic element. There is no reason why operations of this sort should not be of frequent occurrence in nature, and I have no doubt that if we really knew what goes on we would be astounded at the diversity of the trials of this sort that are by chance made in each season of developmental activity.

I know that the type that arose was stable from the experiences in the laboratory and inability to break it up, and its ability to survive was tested at a location created for it at Tepextempa, Vera Cruz, Mexico, where it was placed in tropical savannah conditions in 1908 and without effort passed through four generations in 1908 and 1909, being exterminated in the winter of 1910 by the clearing of the fields for the planting of cane. It was introduced into the deserts at Tucson in 1909 and thrived through two generations in that year, but was killed in the winter of 1909-10 by defects in cage construction which prevented them from penetrating deep enough into the earth to escape the desiccation of the winter months. The entire population was found dead in the spring of 1910 along the wire bottom of the cage. The type could not be tested in nature in any of the northern regions and could not be turned loose in nature, because the food it could eat could not grow and survive the northern winter. I tried it on potato at Chicago in inclosed spaces, and it was completely exterminated, never even making a start at reproduction. It has the capacities, limitation, and characters of natural species—and the experience is suggestive of a method of origin of groups in nature that may prove to be of wide occurrence and productive of much diversity in natural forms.

It is suggestive that the observations show the origin of a group and not of an ancestor, and perhaps for this one reason such origins would on the whole be uniformly more certain of successful competition in the habitat into which it was thrust by the method of its production. This series, it is true, showed only recombinations, nothing in the way of new characters; but other reactions of somewhat similar nature have shown new conditions in the substance, and there is no reason why in similar instances new attributes may not well be the product of reactions like the one observed in the combination of these two species in my experiments.

LEPTINOTARSA DECEMLINEATA × LEPTINOTARSA OBLONGATA.

The crossing of these two species has given further data concerning the gametic composition and reactions different from that derived in the crossing of the other species described in Chapter IV. In Chapter II, I have described these two species, and the chief points of difference, between them at all stages in their ontogeny are shown. There is difference in the form and general type that is measured by the form-index; there are differences of color and pattern in the larvæ, in pattern and color of the adult, in reactions to different environmental agents; but both have about the same rates of development (*Ac* values) and feed upon species of succulent solanums; *L. oblongata* upon *S. rostratum* and its allies; *L. decemlineata* upon either the *rostratum* or *tuberosum* groups. *L. oblongata,* however, does not thrive as well upon *S. tuberosum* as it does upon the *S. rostratum,* and although it can be made to eat *tuberosum,* it always shows a decided preference for its native food. Both are easily grown side by side in the laboratory with perfect success, showing no changes as the result of the enforced captivity, so that crosses can be made under the conditions of the breeding-quarters, and thus to a large extent eliminate the possible complications that external conditions might introduce into the experiments.

The crossing of the species is not a perfectly free one, although it is obtained with relative ease in all of the stocks of these species that I have seen. Some cross more freely than others, and all with greater certainty of success after they have been under the same conditions for several generations and have been subjected to the same routine breeding treatments. There is no entire sterility between them, and infertility seems on the whole to be failure to complete development rather than failure of the two to breed. All of the F_1 hybrids are fertile and have given fertile progeny, so that the operations are not complicated by the problems of sterility or lack of interfertility of F_1. It is true, however, that they are less certain to produce progeny when the stocks are fresh from the original habitat than when both the lines have been long grown under the same conditions.

Under the conditions given as to source of stocks and conditions of experiment, the crossing of these two species is always complicated by the extent to which the cross shows dissociation of the two gametic systems and the number of interchanges of elements that occur. This is evident even in F_1, and in F_2 the complexity is realized more fully when one attempts to work out any of these series of crosses. All of the crosses recorded here are between stocks of *L. oblongata* that originated from the "Temisco colony" near Cuernavaca, Mexico (No. 607 and No. 619), and my stock of *L. decemlineata* at Chicago (No. 99). When crosses are made between these two species, in the F_1 fraternities the larvæ show uniform dominance in color and pattern of the *decemlineata* parent, but in the adults the fraternity shows several well-marked combinations of the parental characters in widely varying ratios. The types commonly found in F_1 are as follows:

(*a*) *decemlineata* in form, with pale yellow-white hypodermal color.
(*b*) *decemlineata* in form, with pronotal color of *decemlineata* and yellow elytral color of *oblongata.*
(*c*) *decemlineata* in form, with yellow-white elytral color of *decemlineata,* and yellow pronotal color of *oblongata.*

(*d*) *decemlineata* in form, no lipoid color in any part, appearing translucent.
(*e*) *decemlineata* in form, but in all other characters *oblongata*.
(*f*) intermediate in form, and showing variable combinations of the characters of the two parent lines.
(*g*) *oblongata* in form, and in all other characters *decemlineata* in aspect.
(*h*) *oblongata* in form, with pronotal color of *decemlineata*.
(*i*) *oblongata* in form, without lipoid color, appearing translucent in color.

There are three chief types that appear, *decemlineata*, intermediate, and *oblongata* in form, all more or less complicated by the appearance of the characters from both parents, so that the F_1 array is most diverse in appearance. This diversity is not limited to appearance, but is shown fully in the breeding of these F_1 types for F_2, which shows that the F_1 fraternity is not a uniform

TABLE 19.

	First group.					Second group.	Third group.			Total.
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	
Fem. obl. × 10-lin. m.....	6	14	7	1	...	29	13	9	...	79
Fem. obl. × 10-lin. m.....	46	4	11	21	6	71	43	11	4	217
M. obl. × 10-lin. fem.....	...	1	...	68	27	92	41	4	...	233
Fem. obl. × 10-lin. m.....	92	14	...	1	4	21	11	74	9	226
M. obl. × 10-lin. fem.....	...	...	1	6	...	141	1	7	...	156
M. obl. × 10-lin. fem.....	11	17	29	31	4	107	92	31	4	326
M. obl. × 10-lin. fem.....	19	11	21	...	1	26	4	4	39	125
Fem. obl. × 10-lin. m.....	11	14	4	41	4	56	28	43	71	268
Fem. obl. × 10-lin. m.....	9	4	...	...	...	12	...	...	6	31
M. obl. × 10-lin. fem.....	...	...	...	1	5	86	1	...	...	93
Fem. obl. × 10-lin. m.....	...	...	...	...	...	147	...	...	...	147
Fem. obl. × 10-lin. m.....	9	5	...	...	...	77	...	81	3	175
M. obl. × 10-lin. fem.....	27	46	...	...	7	5	...	93	181	359
Fem. obl. × 10-lin. m.....	11	...	4	49	17	92	12	18	9	212
M. obl. × 10-lin. fem.....	...	1	...	21	7	171	5	9	3	217
Totals	241	131	77	240	82	1133	251	384	329	
	771					1133	964			2864

heterozygous population at all. It is all heterozygous, but not all in the same manner, so that out of an F_1 array different mated pairs show many differences in the production of F_2 and in the progeny produced. The character of the F_1 arrays is shown in table 19, which is the summation of 15 crosses between these two species under the conditions of experiment, as far as the F_1 arrays are concerned.

The breeding of these F_1 types of heterozygotes shows that there are three main types of action which are in the main coupled with the type that one starts with in F_1. Those in which the *decemlineata* type is dominant in F_1 shows uniformly one type of reaction in this series; those intermediate another, and the set with *oblongata* form dominant still a third set of reactions in the production of F_2 and subsequent generations, which are in the main centered about the reactions of the form-producing agents, and little or not at all concerned with the color and pattern agents that may be present.

BREEDING OF F_1 HETEROZYGOTES.

The chief point of interest in this series is the extent to which there has been dissociation of the gametic systems with interchange of agents or groups thereof. All of these F_1 fraternities are so complex that in no single instance have I had the aid and space for a complete analysis of the entire condition presented in one fraternity which would involve in F_2 and F_3 hundreds of simultaneous matings to adequately test the total complexity of the interchange of agents. I have, in the main, limited my efforts to the breeding and testing of types and lines that showed points of interest with regard to certain gametic agents and their capacity for change in position and their action in the new positions. The complication, or diversity, is due to the numerous color and pattern determiners that suffer interchange in crossing and the numerous possible combinations thereof. Eight pairs of character groups capable of metathesis are concerned.

WITH THE DECEMLINEATA FORM DOMINANT.

As shown in table 19 diverse types come out in F_1 with *decemlineata* dominant in form, and of these, (*a*), that is *decemlineata* in appearance, has given some

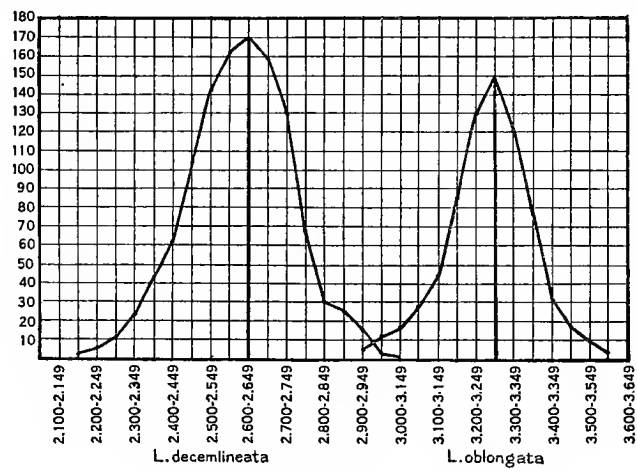
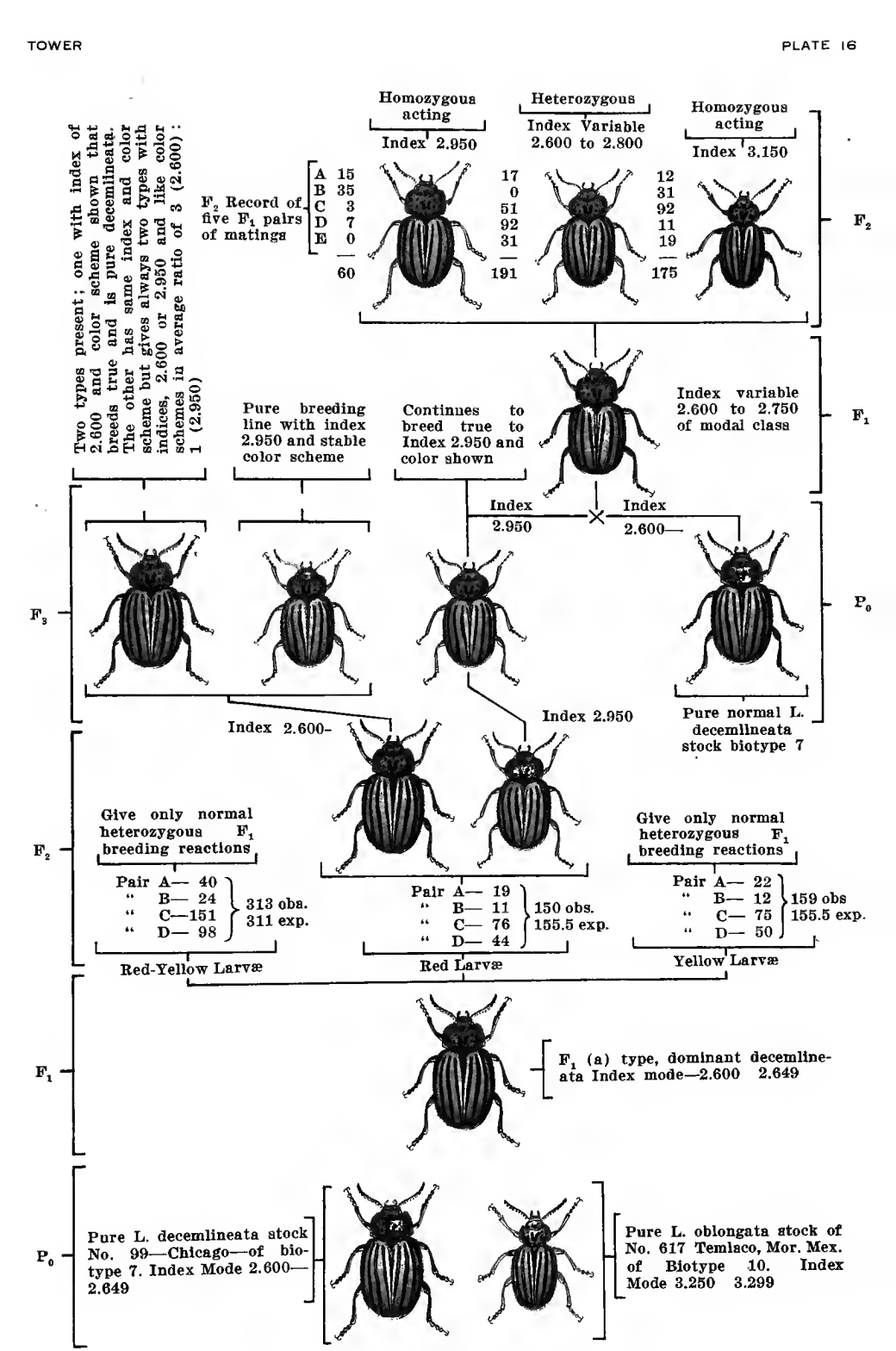


FIG. 10.

interesting results. The frequency of this type in F_1 is shown in table 19, in which it appears that the occurrence is highly variable, may even be absent or present in the population in considerable numbers, and none that has been tested breed true. In figure 10 I have shown the determinations of the form-index in the two parental lines. In *L. decemlineata* the mode and mean of the index falls upon the class value 2.600–2.649, with a normal distribution on either side. In *L. oblongata* the mode and mean fall in or close to the class value 3.250–3.299, also with even distribution on both sides. There is little or no overlapping of the index-curves in these two stocks; the form-index values are constant, and little trouble is encountered in separating them by inspection in


Homozygous acting
Index 2.950
Heterozygous
Index Variable 2.600 to 2.800
Homozygous acting
Index 3.150
F2 Record of five F1 pairs of matings
A 15 17 12
B 35 0 31
C 3 51 92
D 7 92 11
E 0 31 19
60 191 175
F2
F1
Index variable 2.600 to 2.750 of modal class
Two types present; one with index of 2.600 and color scheme shown that breeds true and is pure decemlineata. The other has same index and color scheme but gives always two types with indices, 2.600 or 2.950 and like color schemes in average ratio of 3 (2.600) : 1 (2.950)
Pure breeding line with index 2.950 and stable color scheme
Continues to breed true to Index 2.950 and color shown
Index 2.950
Index 2.600—
P0
F3
Index 2.600—
Index 2.950
Pure normal L. decemlineata stock biotype 7
F2
Give only normal heterozygous F1 breeding reactions
Pair A— 40
" B— 24
" C—151
" D— 98
313 obs.
311 exp.
Red-Yellow Larvæ
Pair A— 19
" B— 11
" C— 76
" D— 44
150 obs.
155.5 exp.
Red Larvæ
Give only normal heterozygous F1 breeding reactions
Pair A— 22
" B— 12
" C— 75
" D— 50
159 obs
155.5 exp.
Yellow Larvæ
F1
F1 (a) type, dominant decemlineata Index mode—2.600 2.649
P0
Pure L. decemlineata stock No. 99—Chicago—of biotype 7. Index Mode 2.600—2.649
Pure L. oblongata stock of No. 617 Temlaco, Mor. Mex. of Biotype 10. Index Mode 3.250 3.299

SHOWS IN GRAPHIC FORM RESULTS OBTAINED IN SOME TEST REACTIONS IN CROSSING *L. decemlineata* AND *L. oblongata*.

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a fraternity of hybrids, doubtful cases being measured and tested by breeding; the two methods combined give certainty of operation.

When the F_1 apparently entirely dominant *decemlineata* is inbred the larvæ produced from such matings show pure reds, reddish yellow, and yellow in proportion based on the scheme of 1 red: 2 red yellow: 1 yellow, although the individual fraternities are highly variable. The adult progeny produced from these groups is complex, and only that from the red line of larvæ is of interest here, the others not being presented. The red larvæ produce adults that are in pattern and coloration pure *decemlineata,* but show in form-index wide variation and a bimodal polygon with two modes, one on 2.600, the other on 2.950. Matings from these modal classes develop from the 2.950 form a pure line that is stable at once for the character of form-index and uniform for other *decemlineata* characters. The group that centers about the value 2.600 is variable, some giving pure-breeding lines, others showing wide deviation or bimodal conditions, but in all the numbers are not constant and the ratios are not in harmony with present neo-Mendelian ratio systems.

When a race is isolated that has the form-index 2.950 as its constant modal value and is crossed with pure *decemlineata* from the parent strain, the product of the cross in F_2 is the production of an array all *decemlineata* in character that shows a bimodal condition, with the exception that the form-index values of the modal classes of each modal group are different; namely, 2.600, 2.600–2.800, 3.150. The appearance of these types is erratic and the fraternities show diversity in the numbers presented, but from the modes 2.600 and 3.150 pure races are derived. Two of the modal groups are apparently homozygous, but the other contains individuals which when mated give pure-breeding lines with the form index 2.600, while others are heterozygous. I have presented this series of reactions in plate 16, and also data of some fraternities showing the irregularity of the array with respect to the numbers present.

The two lines that originate from this F_2 array give two different form-types of an organism that is *decemlineata* in all other respects, and I have not been able to derive from either of these lines any characters that indicate the presence of the *oblongata* parent. The race developed with the modal value of 3.150 is perhaps the most interesting to us in that in all respects save form it is typical *decemlineata,* even to minor habits and reactions, as well as in color and pattern characters. The odor of its secretions are characteristically *decemlineata* and not those of *oblongata,* which, even to my senses are recognizably different.

It is probable that we are dealing in this race with one of two possible conditions; either that the line is the result of the transfer of all the commoner *decemlineata* qualities onto an *oblongata* form-base, or that the form-determiner of *oblongata* has been dissociated from its complex and replaced in the pure *decemlineata* complex, the normal agent for form determination of that species. The latter seems the more probable, and indication of its probability is found in the change produced in the pronotal pattern of the race, which gives an appearance much like that found in pure *oblongata.*

Testing of this race by crossing with both of the parent species has given suggestive confirmation regarding its constitution. When crossed with *oblongata,* the product in F_1 is intermediate between the two in all respects. For this

test I have used only pure white, biotype 10 lines of *oblongata.* F_2 in the test shows an array that is suggestive only of a monohybrid reaction, the *oblongata,* the tested type, and an intermediate coming out in nearly perfect ratios (table 20) showing that the types in the test have acted as units, neither gametic system having been broken or interchanged agents. This test is of interest in that it shows that the race does not have in it the characters of *oblongata* in at least easily available form, else there would have appeared in F_1 of this combination, or at least in F_2, some indication of them. As a test reaction this is only suggestive, not in any way final.

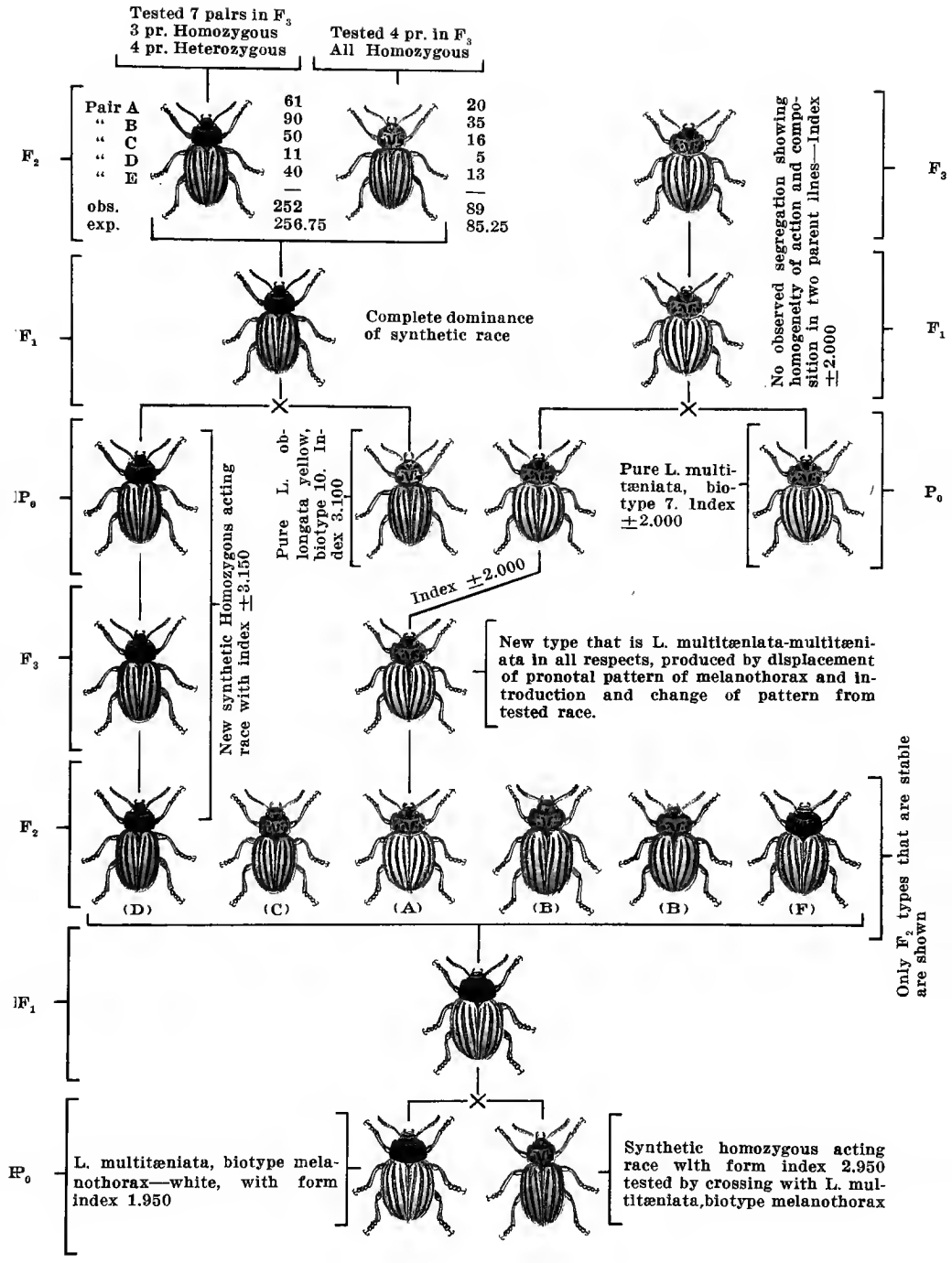
When crossed with the *decemlineata* parent stock, the product in F_1 is an intermediate array, and in F_2 there appear the three types of form-index values, as shown in figure 11. The reaction seems to have been of the simplest type possible, in that the two gametic systems acted as units and suffered no change in the passage through the F_1 hybrids.

TABLE 20.

	Decemlineata type.	Intermediate type.	Oblongata type.	Total.
Pair A, F_2	20	47	19	86
Pair B, F_2	37	74	36	147
Pair C, F_2	12	3	9	24
Pair D, F_2	13	15	11	39
Pair E, F_2	20	61	27	108
Pair F, F_2	16	33	15	64
Observed	118	233	117	468
Expected on basis of monohybrid reaction.	117	234	117	468

A more conclusive test was made by crossing the type with a line of *L. multitæniata* biotype *melanothorax,* that was known to be pure for form-determiner of a value of 1.1950, and with only the pronotal pattern of *melanothorax* present. This combination showed dominance of the *melanothorax* type in F_1, but in F_2 there appeared a complicated array that gives indication of the nature of the special race investigated. This I have shown in schematic form in plate 17.

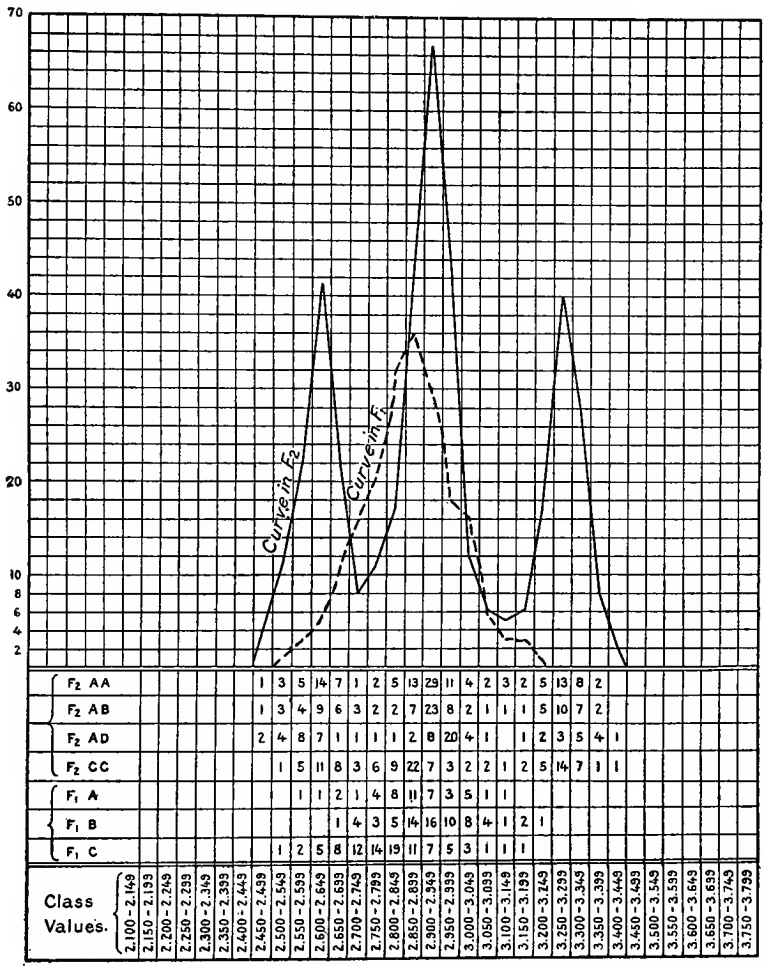
The array given in plate 17 does not show the entire complexity of the F_2 product, which is further complicated by juvenile stages and groupings of adult characters, but shows the essential adult conditions that are present in F_2, indicating what the suspected race was composed of. An interchange of the characters takes place, such that the F_2 shows new combinations and types not present in the original lines. Most striking is the occurrence in F_2 of a biotype 7 *multitæniata* type that is the product of a synthetic combination between the pattern system of the pronotum of the tested type and the *melanothorax* stock, in which the pronotal pattern systems have been interchanged, giving as a result the type shown in F_2 of plate 17 (*A*). The pattern type of the tested line is that of *oblongata,* in which there are present all of the elements that occur in *multitæniata,* but in different relation. There also is obtained the *decemlineata* type (*B*), the *tested* type (*C*), *melanothorax* type (*F*), tested type with *melano-*


Tested 7 pairs in F2
3 pr. Homozygous
4 pr. Heterozygous
Tested 4 pr. in F2
All Homozygous
Pair A 61
" B 90
" C 50
" D 11
" E 40
obs. 252
exp. 256.75
20
35
16
5
13
89
85.25
F2
F3
F1
P0
Complete dominance of synthetic race
No observed segregation showing homogeneity of action and composition in two parent lines—Index ±2.000
Pure L. oblongata yellow, biotype 10. Index 3.100
Pure L. multitæniata, biotype 7. Index ±2.000
Index ±2.000
New synthetic Homozygous acting race with index ±3.150
New type that is L. multitænlata-multitæniata in all respects, produced by displacement of pronotal pattern of melanothorax and introduction and change of pattern from tested race.
(D)
(C)
(A)
(B)
(B)
(F)
Only F2 types that are stable are shown
L. multitæniata, biotype melanothorax—white, with form index 1.950
Synthetic homozygous acting race wlth form index 2.950 tested by crossing with L. multitæniata, biotype melanothorax

GRAPHIC PRESENTATION OF TEST REACTIONS IN *L. multitæniata*, SHOWING THE ACTION OF CERTAIN FORM DETERMINERS.

JULIUS BIEN LITH. N.Y.

thorax coloration (*D*), and others that breed true, and also combinations that are hybrid and not shown. The point of interest is the showing that a form-determining agent is present, acting, and capable of change, and the interchange of the pattern systems of the pronotum.



Class Values	F2 AA	F2 AB	F2 AD	F2 CC	F1 A	F1 B	F1 C
2.100 - 2.149							1
2.150 - 2.199			2	1			2
2.200 - 2.249		1	4	5	1		5
2.250 - 2.299		3	8	11	1		8
2.300 - 2.349		4	7	8	2	1	6
2.350 - 2.399		9	1	3	1	4	12
2.400 - 2.449		6	1	6	4	3	14
2.450 - 2.499	1	3	1	9	8	5	19
2.500 - 2.549	3	2	1	22	11	14	11
2.550 - 2.599	5	2	2	7	7	16	7
2.600 - 2.649	11	7	8	3	3	10	5
2.650 - 2.699	8	23	20	2	5	8	3
2.700 - 2.749	7	8	4	2	1	4	1
2.750 - 2.799	1	2	1	1	1	1	1
2.800 - 2.849	2	7	1	2		2	
2.850 - 2.899	5	2	2	5		1	
2.900 - 2.949	13	2	3	14			
2.950 - 2.999	29	1	2	7			
3.000 - 3.049	11	1	1	3			
3.050 - 3.099	4	1		1			
3.100 - 3.149	2		1	2			
3.150 - 3.199	3	1	2	5			
3.200 - 3.249	2	1	3	14			
3.250 - 3.299	5	5	5	7			
3.300 - 3.349	13	10	4	1			
3.350 - 3.399	8	7	1	1			
3.400 - 3.449	2	2					
3.450 - 3.499							
3.500 - 3.549							
3.550 - 3.599							
3.600 - 3.649							
3.650 - 3.699							
3.700 - 3.749							
3.750 - 3.799							

FIG. 11.

The existence of the form-determiner is shown by the derivation from the F_2 array of *decemlineata* forms that are approximately the result of the combination of the basal form-factor of the tested race and the form-determiner from the *melanothorax* type, which result could only be produced in this test by the transfer of the form-determiner from the *melanothorax* type to the tested type, and the result is precisely that which is expected.

This test is in all respects a routine Mendelian reaction, uninteresting, excepting in the ability which it gives to test the nature of the agents that are being

dissociated and recombined in the crossing of these two species. In this race, the subject of the test, there is clearly present in the interactions two groups of agents, or two agents, a form-factor group that is not dissociated and a form-determiner, the interaction of the two being to produce the characteristic form or type as measured by the form-index, or recognized by inspection in many instances in its pattern modifications. These two groups of agents are purely symbolizations at present and no indication is to be had that shows what they are.

The race tested is in all respects *decemlineata,* with the exception of form, which is that of *oblongata,* while its breeding true shows that there has been formed a permanent combination, that is, a harmoniously acting one, so that it gives uniform fraternal arrays, generation after generation, the usual test of purity and stability. That the race is due to the combination of the *decemlineata* complex with the form determiner from *oblongata* is shown in the test cross with *melanothorax* in which, in the complex interchange that takes place, there comes out a *decemlineata* form as the result of the combination of the *decemlineata* form-factor complex with the determiner from the *melanothorax* race. This result could only have come about in two ways; either it is the product of the process stated, or the production of the *decemlineata* form in F_2 is due to the presence in the tested line of the *decemlineata* determiner being present in hidden form in the tested race and not acting. However, tests in crossing with the pure *decemlineata* and *oblongata* have failed to show the existence of it in reactions where it would have been revealed if present. It is hardly to be expected that the race that is being tested is due to the transfer of its very numerous *decemlineata* characters to an *oblongata* base. There are too many of these and too decided an absence of *oblongata* attributes in the combination, nor is the combination precisely the *oblongata* parent condition, but below it, towards *decemlineata,* showing that the combination does not react in the same manner as in either of the parental stocks. The fact that the line is in color, reaction, odor, habits, development, and in all respects *decemlineata,* save in the element of form which is nearly that of *oblongata,* the experiences in the tests of the line that have been made force one to the conclusion that there has been produced a combination of agents from *oblongata* with the *decemlineata* complex, producing a new, homozygously acting genetic line. The series shows, I believe, the dissociation and recombination of the form-producing agents in the gamete and the existence of two groups, the interaction of which is necessary to the production of the specific end-result, a form-factor group, and a determiner group, and the evidence shows that it is the determiner group that is transferred and recombined and not the factor series. It is further shown in the test made with *melanothorax* that the pronotal-pattern series are capable of interchange as a whole, another example of the action as a whole in crossing of meristic series of characters, other examples being the behavior of the larval patterns in this as in other series of crosses already described.

Three matings of the (*a*) type in F_1 have given different results from those presented, and have not shown the presence in F_2 and in later generations or in tests of the breaking up of the line into the different form-types shown in the series just described. These three lines have been uniformly *decemlineata* in form-index and showed only the production of diverse F_2 groupings dependent upon the metathesis of different agents that came from the parent stocks, espe-

cially color-characters. Each of the three lines has been bred out to at least F_5 or beyond, and no breeding or testing has been able to break them up into different form-index groupings, the *decemlineata* form remaining stable and constant.

At no point in the crosses of these two species has there been any linking or correlation of the different color and pattern characters with these form-producing agents, and so in the three pairs that breed true for the *decemlineata* form there was abundance of combinations of the color and pattern conditions. The findings suggest the existence either of another instance of a masked heterozygote for form with the *decemlineata* dominant, or the obliteration of the *oblongata* form and other agents in the F_1 combination. In crossing natural species it has often seemed that the results of the interactions in F_1 have been to produce a total obliteration of some of the agents of one or the other parent, as far as further ability to get them out of the combination is concerned. Whether they have actually ceased to exist as agents, having been thrown out of the complex produced in F_1, or are held in fixed form not manifested or manifest in new actions, is a problem for further investigation.

About 60 per cent of the F_1 (*a*) type *decemlineata* matings are heterozygous in the ordinary sense and show in F_2 only routine combinations of the different characters and groups thereof that are dissociated from each of the two parental complexes. From these, extracted types of each of the parental species as well as many stable combinations are commonly derived, while other rarer combinations and associations are erratic in their appearance.

WITH THE FORM INTERMEDIATE.

In every cross between these two species there are certain intermediate forms as regards form-index, with variable associations of visible parental characters. Breeding of these shows in all that have been tested that they are uniformly typical F_1 heterozygotes, and the F_2 arrays that are produced from them show only routine separations into the parental form-types with differing combinations of the color, pattern, and juvenile characters, so that out of the series one can obtain a very considerable number of stable combinations of the characters shown in the two species. The F_2 arrays are in all instances irregular and the ratios are not in accord with the expected arrays, owing to the chance non-dissociation of two or more characters in one or both parents. At no point in the series is there any indication of blending of these characters, and the reactions are always clean-cut and precise, the uncertainty being entirely inability to devote enough space and labor to any one series to fully analyze its composition.

These F_1 heterozygotes are interesting because of the irregular metathesis of one or several characters which at other times or even in the same lines are dissociated, and suggests agents present either within or without that act to influence this association of factors and the subsequent behavior of them as a unit. The direct result of this condition is that one can derive a large number of races from the cross, which breeds true—*i. e.*, is homozygous acting in genetic lines—and these when crossed give simple Mendelian arrays, usually, those characteristic of monohybrid or dihybrid reactions. Some notion of the extent of the array that can be obtained is shown by the series of pairs of characters that

have been found to interchange in the crossing of these two species. The commoner are shown in table 21.

TABLE 21.

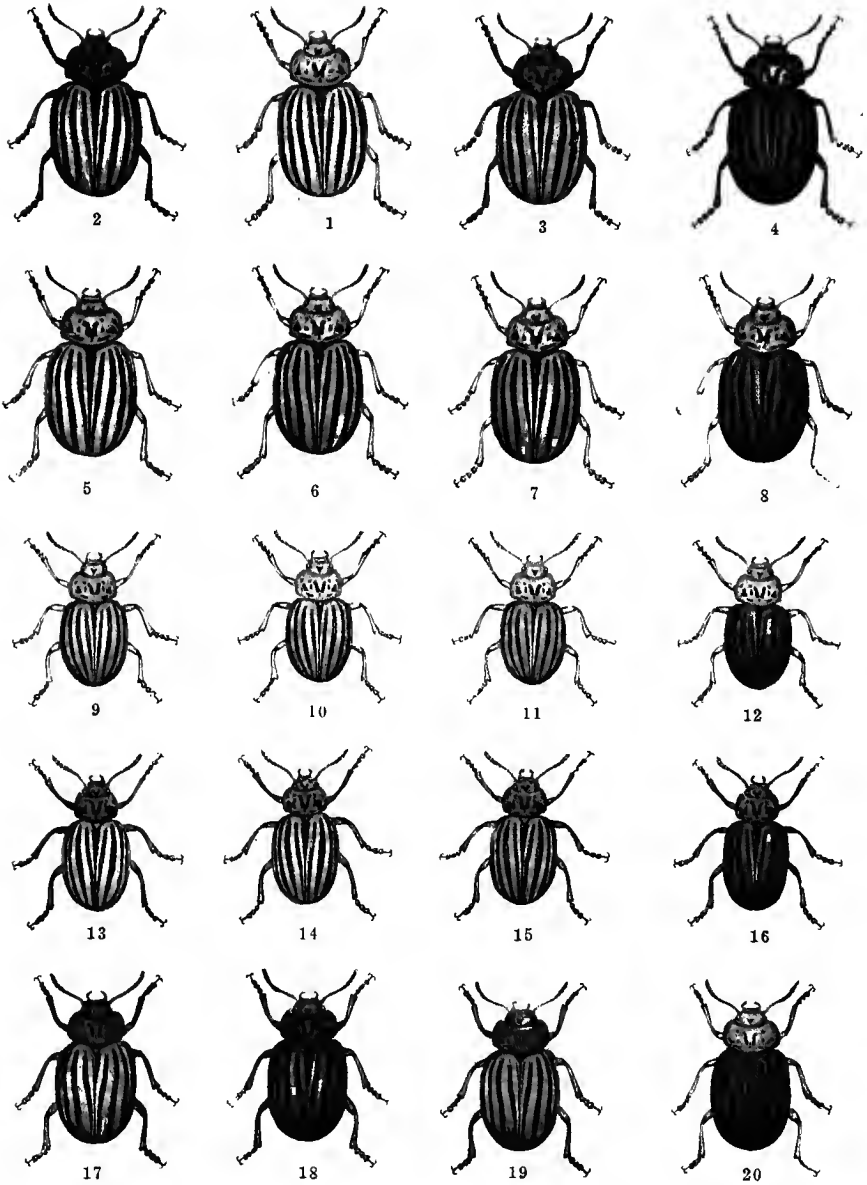
Character.	Decemlineata.	Oblongata.
Form determiner	Fd_3	Fd_4.
Larval body-color	R	Y.
Larval pattern-system..	[($Pd_1 + Pd_2 + Pd_3$) (10 L)]	[($Pd_1 + Pd_2 + Pd_3$) (ob)].
Elytral color	Yw	Y.
Pronotal color	YR	Y.
Species base	decemlineata	oblongata.

These six pairs of agents, which are recognizable as being shifted in crossing, give, if they act independently, a possible hypothetical F_3 array as follows:

(1) Number of individual combinations..... (4^n) or $4^6 = 4096$.
(2) Number of terms in the series..... (3^n) or $3^6 = 729$.
(3) Number of stable combinations..... (2^n) or $2^6 = 64$.

To this series could be added the combinations of some of these into associations that act singly, and also the dissociation of some of the groups into component simpler ones. Thus, in color of the body there has been obtained no hypodermal color, white, pale yellow, yellow, chrome yellow, orange, red, and vermillion in pure-breeding strains out of this cross, and localized sometimes in the pronotum, sometimes in the elytra, or at others uniform over the body as a whole; these, of course, in homozygous-acting races that behave when crossed with other races as pure Mendelian pairs. I have not the least doubt that if time, space, and labor were available one could easily obtain from this cross several hundred of pure-breeding lines, each distinctive in one or more characters, alternative to one another throughout.

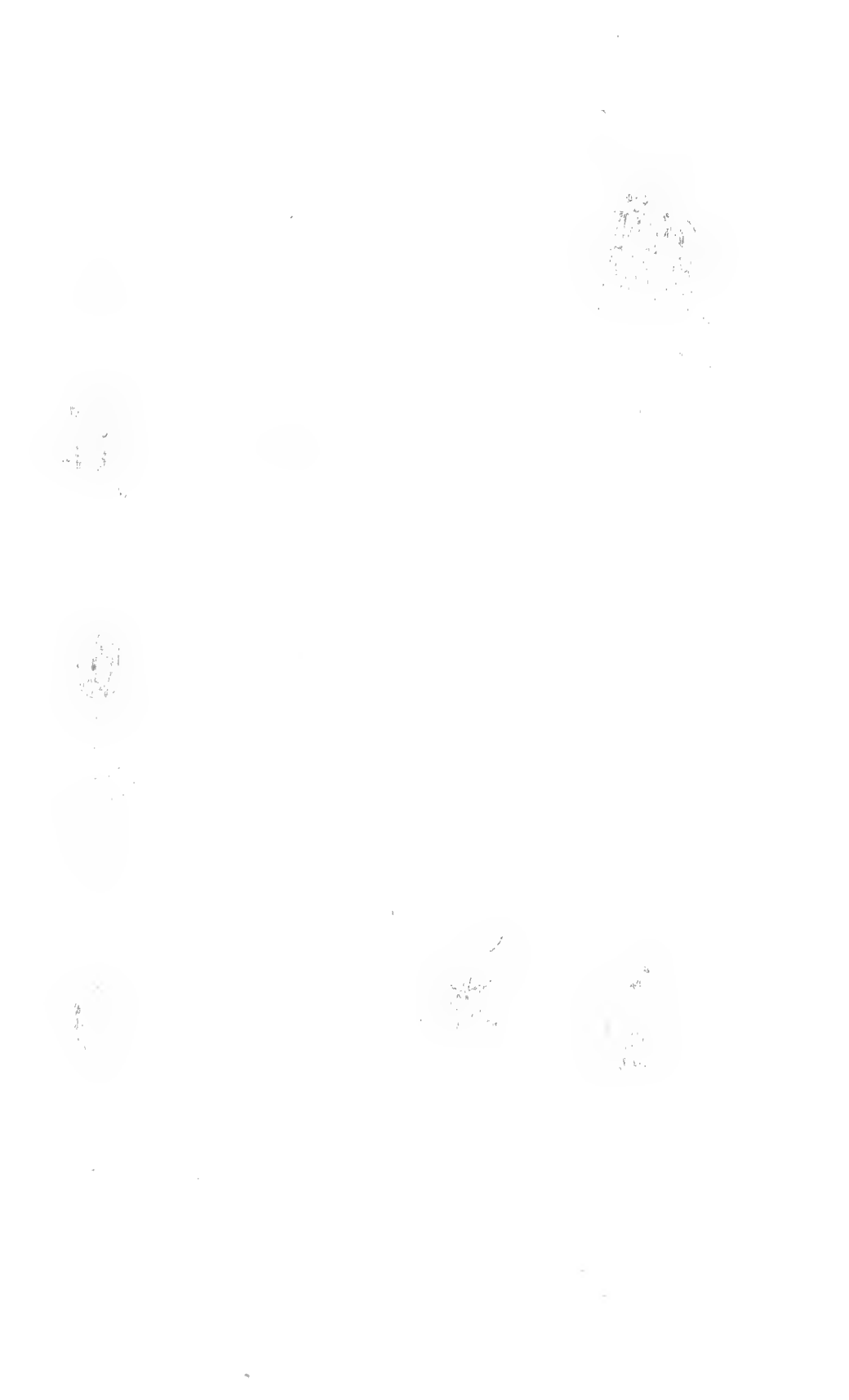
Two points of general interest come out in this complicated reaction: (1) the dissociation of each of the gametic systems and the numerous interchanges that are shown as the result of this cross; (2) the action of agents and groups of agents as units, irrespective of the number of contained agents present. This second point is of especial importance as showing that the type of reaction is not one that involves the ultimate nature of the interchanging agents. The entire series of reactions, the numerous stable end-products, presents a condition that is strikingly in contrast with that shown in the crosses of species described in the previous chapter. In plate 18 I have shown some of the homozygous biotypic lines that have been derived as the products of the intercrossing of these two species. Those shown are all derived from the middle group of F_1, as far as the complications presented in the adult condition are concerned, and consist only of a few combinations of form and color. When these are added characters in the juvenile stages, very many lines, each with its own sequence of characters, are possible of derivation from this cross. The details of the production are not worth presenting, involving only routine operations, with interplay of external agents for the attainment of certain end-results. Each one of the 20 conditions shown in plate 18 is biotypic for its adult characters and with ease can be made biotypic for its entire constitution. The 20 shown can be made 40 by change in larval color alone, yellow and red larval races being possible of each one of these, and so on the biotypic lines could be multiplied.



K. TODA DEL.

JULIUS BIEN, N. Y.

This plate shows graphically some possible combinations that can be made in *L. multitæniata* after pure biotypic lines have been established for these characteristics. In this instance body-color of red, yellow, and light yellow and body-form are concerned, giving the array of types shown in the figure, all of which have been actually produced and found to breed true for at least two generations as extracted types. Many of these have been bred true for 6 to 8, and even 10, generations in biotypic pure lines.



WITH OBLONGATA DOMINANT.

The third F_1 group in the primary cross of these species shows dominance of *oblongata,* with relatively few complications. None of them is pure, all that have been tested being the heterozygous, but in varying degrees and directions. From the third F_1 group many lines of homozygous-acting combinations have been derived, of which the most interesting are those that involve the form determiners, and a duplicate series shown for the first group is found, showing that the form-determiner is dissociated and interchanged in all F_1 interactions. The series, however, gives nothing in action or principle that differs from that seen in other aspects of this species cross.

One of the chief points of interest in the crossing of these species is in the relation of the two gametic systems in F_1. In F_1 there appears an equivalence and blending of the two when the cross is not complicated by certain agents within and without, whose action has been analyzed. As shown in table 17, the F_1 product under the conditions of the laboratory quarters is highly diverse, even when the parent lines have been fully accustomed to these conditions for generations. There is, of course, not constancy in this environic complex, but rhythmic regularity of conditions which diurnal changes produce in the organisms—possible differences in physical condition that may be in some measure productive of the complex F_1 array and the amount of the dissociation of the gametic systems shown. That the conditions of the medium do have something to do with the complexity of F_1 is shown by the crossing of these species under the mean conditions of the breeding-quarters, in special apparatus, but constant, when there is produced in F_1 only the blended intermediate type with uniformity in the entire array in all characters; but in breeding these are all strictly heterozygous, and in F_2 give heterogeneous arrays, depending upon the conditions present in the medium.

The complete analysis of this cross would require many times the facilities that I have had for controlled conditions, but enough is given concerning the cross of these two species to show that the complexity of the products of the cross are dependent upon the condition of the materials and the oscillation of factors present in the medium. The crossing of these two should first be worked out under constant conditions, and then the rôle of departures and changes in the medium could be determined. The cross shows how, in the ever-changing conditions in any natural habitat there would be produced many possible combinations and rearrangements, giving many distinct lines of descent. It is this operation in nature that has much of interest, because of its possible bearing upon the production of heterogeneity in nature, and also because the same two species have in nature been utilized in experiments in the production of mutating stem-forms.

Crossing of these species under the usual conditions of life produces what seems to be an extensive disruption of both of the gametic systems, which is in experience even more complicated than here indicated. Not only are the unimportant characters of color and pattern dissociated and interchanged, in differing aggregations, but habits, food-relations and growth are involved. As a result, hardly any two cultures are alike, and derivation of homozygous-acting lines is often attended with endless difficulties; nevertheless, at no point has there been anything which on test proved not to be regular in its action and constitution. I wish that it were possible to present at this time even an approxi-

mately complete account of the analysis of the intercrossing of these two species, but the complexity of the dissociation has made it largely a practical matter of inadequate facilities.

Both of the species are stable and constant and even under extreme changes of the environment they retain their stability, altering only characters if at all, but in combination in crossing the lines that have been experimented upon show no end of diversity. Constant conditions surrounding the cross reduce or eliminate the dissociation of the gametes, the reaction appearing as simple, but with ranging conditions in the medium the disruption is extreme. It may be objected that in reality only a few character agents are involved, and this may be true; but there are some agents, that are specific and localized, capable of separation and recombination through the reactions of crossing plus the conditions of the medium, so that it makes little difference what is personified or symbolized to express our experiences. The important point is that these unseen agents react precisely and orderly. In this series there are many color-conditions, especially of the hypodermal colors, that are unstable and break down at death, but which are nevertheless distinct and precise in life, and react as distinct contrasting units of operation. I am aware that the color-producing molecules may well be the same in all, and that the color differences may be only differences in the arrangement of the component atoms and atom groups of the molecules; but there is some precise cause of this.

The result of the crossing of these two species under ordinary conditions may be characterized as a collision between them, with disruption of each of the gametic systems into fragments of different sizes and composition, which are in form and position to be built into new positions and arrangements, so that the results of a cross of this order might be the production of many different lines of descent. It is the antithesis of the reaction of *signaticollis* × *diversa,* where the systems were not disrupted at all. The experience is suggestive of what might conceivably happen in nature and be productive of some of the groups of related species that occur, where lines of demarcation are wanting or difficult to draw. It would be impossible to decide whether in any instance in nature where there was extensive heterogeneity it is due to this or some other cause, for the reason that the production of the condition would soon pass away, leaving behind only the heterogeneous population, in which in nature nothing is known as to the relations in breeding or in genetic reactions of the component elements. I have put this to test in experiments described in a later part of this report.

Whether all cultures of *decemlineata* crossed with all cultures of *oblongata* would give this result I do not know. I have tested only crosses of my 99 *decemlineata* stock and *oblongata* from Temisco and Cuautla. They may well be local differences in these species, such that the reactions of other sources of materials would not be as complex as I have found them, or might be more so. Whatever the solution of this problem may be, the relation between the two species will not be any simpler than I have found it, and probably is much more complex, taking the species as a whole, so that the potentiality for heterogeneity between these two is very great.

This cross reminds one strongly of some of the events that may have happened in the production of the diverse strains of domesticated plants and animals. Many garden fruits and flowers, dogs, poultry, and pigeons show endless combinations of character that are present in the wild originals; and also

numerous accentuations and developments of these, with few apparently new characters. There have been in domestication crossings without number, control, and perhaps all possible combinations, so that it may be probable that the initial impetus given to primitive man to breed and utilize some of these then wild organisms came from this same sort of initial disruption of two originals, by chance giving combinations pleasing to the savage fancy, so that they were perpetuated with his crude breeding operations, and come down to later periods in culture. The initial cross would in all probability not be intentional, but the product of breeding of the tamed with the surrounding wild forms, as is known to be the case in savage or barbarous establishments.

One point further is of interest in this cross, namely, the fact that the initial F_1 and F_2 arrays are on the whole the most complex, and that thereafter the reactions of the products of the primary cross are all increasingly towards simplicity, as the lines are reduced progressively to homozygous-acting biotypic strains, between which crosses give only the most orderly and monotonous Mendelian reactions and products. This experience is also in harmony with the crossings of domesticated plants and animals, to which, thus far, the majority of the orderly and fully analyzed Mendelian operations are confined. Thus, the 20 types shown in plate 18 are, when crossed in biotypic condition, productive of monohybrid or dihybrid reactions, rarely of trihybrid, while the huge majority in the tests thus far are of the simpler types. In many respects this cross of *decemlineata* and *oblongata* shows the difference between species or groups in nature and the purified races of cultivation in the complexity of the natural, the homogeneity of the cultural, in reactions that test their gametic constitution; but in both there is no difference in the basic principle of reaction. This is the essential point and one upon which attention is directed in the summary at the close of this chapter.

LEPTINOTARSA DECEMLINEATA × LEPTINOTARSA MULTITÆNIATA.

The crossing of these two species is complicated and can best be presented under two headings—the crossing of the natural species and the crossing of biotypic lines. These species, especially *multitæniata,* are in natural conditions complex in their constitution, and when they are crossed the ensuing F_1 and F_2 arrays are diverse, depending upon the original composition of the stocks used. In some of the earlier chapters it has been shown that for *multitæniata* the composition is complicated by differences in form, pronotal pattern, sports, and other means of gametic complexity. As a result the crossing gives in each set of matings differences and arrays depending upon the stocks used. I have for the purposes of this analysis purified the parent stocks used, and then utilized them in crosses in the determination of the relations between these two species when crossed. I shall describe the results of crossing the biotypic lines first, and then the general reactions when the crosses are made between the stocks as a whole.

CROSSING OF BIOTYPIC LINES IN DECEMLINEATA AND MULTITÆNIATA.

The simplest and standard cross between these two species is that made between a biotypic line of pronotal pattern biotype 7, mean form-index, mean yellow-white pronotal color, and mean pronotal color, each with the pure nonvariable larval characters proper to each species. Two biotypes of this sort are

easy to derive from the parent species, are perfectly homozygous in action, but must be tested by intraspecific crossings to determine the presence of non-manifested gametic contaminations. These when found must be eliminated. Two such lines reared under the same conditions of the medium for several generations when crossed give a relatively certain test of the reaction proper to the two gametic systems, independent of the conditions of the environment, and not complicated by nonhomogeneity in the stocks.

In the crossing of these two species I have used only pedigreed materials that had been reduced in cultures to a biotypic condition, and my crosses have been between the *decemlineata* stock from Chicago (No. 99) and *multitæniata* from Chapultepec (No. 543), Puebla (No. 541), and Texcoco (No. 547) in Mexico. Of this species I have used the pure *multitæniata* biotype 7 and *multilineata* biotype 12. All of these have been of the typical yellow hypodermal color, so that the crosses have not been complicated by the array of hypodermal colorations found in the previous cross. The two species are also not greatly different in form-index, except for the narrow form that is present in the biotype *multilineata* and the broad index that is associated with *tacubayœnsis*.

After these two species have been reared in the same conditions in the laboratory at Chicago for three to five generations and then crossed, the uniform result is the dominance of *decemlineata* in F_1 in the juvenile stages, especially in larval body-color, the patterns and larval-pattern sequences being the same in both. The dominance of the *decemlineata* in F_1 can in many respects be altered, by the use of desiccation, high temperatures, and other agents, to diminish the intensity of the F_1 red, but in no instance has the color been modified to more than an intermediate condition of yellowish red. The F_1 adults show rather uniformly the equal blending of the adult characters of the two parents when the parent lines are in both biotype 7 in pronotum pattern. The pronotal hypodermal color may be variable, swinging between the two parental types, but in the main it is intermediate between them, while the elytral colors are not sufficiently different to produce any change. The form-index, however, shows complete dominance of the *decemlineata* type, with relatively little oscillation and *decemlineata* is the dominant type.

Inbreeding these F_1 for the next generation shows that on the whole the two have been through the cross with no dissociation of the characters, there appearing in the adults in F_2 three classes that are by inspection clearly *decemlineata*, heterozygotes, and *multitæniata*. Some fraternities show this separation with remarkable distinctness, others show the presence of intergrading conditions in the heterozygous series, so that the separation of the entire array can not be made by either inspection or measurement, but in such arrays no difficulty is experienced in selecting by inspection the extreme conditions that always breed as extractives, or of the centrally placed heterozygotes. Other conditions in the array are not so easy of calibration and often are only properly placed after test by breeding. This difference in F_2 in different fraternities is a product of the conditions surrounding the cross, which in general seem to produce, when constant and with little or no oscillation, sharply marked F_2 arrangements; but when the conditions are oscillating the groups are less clearly defined or often obscured entirely by the range in the heterozygous members of F_2.

In these reactions it is certain that the characters of the adult have not been dissociated in passing through the F_1 reactions, coming out of the operation in

original form and arrangement, so that the reaction, as far as the adult conditions are concerned, presents only the array of a simple monohybrid type as far as the crossing of these two biotypic lines are concerned.

In the larvæ the arrays presented are complicated by the unstability of the larval body-colors when in heterozygous combination, so that F_2 always presents two classes, reds and yellows, in variable numbers.

This is entirely the product of the potentiality possessed by the heterozygous members of the fraternity for range in the characters presented, and is in the main, if not entirely, a product of the conditions of the medium or other conditions of life and of purely transient nature. When these fraternities are reared under constant conditions the F_2 array of larvæ shows yellows, yellow-reds, and reds, in close approximations of 1 : 2 : 1.

In nature and under the usual conditions of operation, the F_2 array in its heterozygous members is able to range widely in both directions, so that the result is to produce the curious ratio of an apparent 1 : 1. The homozygous yellow is always more easy of separation than the red, which even in the pure stock is variable in its intensity, depending upon conditions of nutrition and surroundings, so that separation of F_2 fraternities is by no means easy and is complicated by the action of heterozygous individuals, which tend to swing to one or the other extreme of the possible series, giving either reds or yellows, and few uncertain intermediates under ordinary conditions. The F_2 heterozygotes in all of these experiments are more sensitive to the action of the external conditions, as shown by their ability to fluctuate between the possible extremes. This fluctuation of the heterozygous individuals is by no means common in described crossings, largely due to the absence of observations upon the juvenile stages of organisms in crosses. Newman has recorded variations in the aspect of the embryos of *Fundulus,* and I have seen in many of the materials that have passed through my hands this same fluctuation in the juvenile characters in ontogeny, and have experimentally altered them one way or the other by change of conditions or food. These changes are thus far in my experience entirely without genetic significance, although they show in the heterozygotes susceptibility to external conditions not shown in the homozygous types, and further these are always far more strongly manifested in the juvenile stages than in any of the adult characters where fluctuations of this sort are not common.

The evidence from this cross of the mean biotypic lines of these two species shows that the two gametic systems act in crossing as unit systems coming out of the cross in totality, without interchange of the characteristic of either parent, so that the reaction in the cross is of the monohybrid type. This result is true only of the condition in the stocks used and under the conditions of experiment given. Further, in experience with the cross the regularity of the reaction is increased with the approach to constancy in the conditions of the medium, the sharpest reactions being observed in controlled series, where the conditions were constant and at the mean of the environmental complex present in the breeding-quarters antecedent to the time the stocks were crossed.

These two modal biotypes represent the species complex devoid of any accessory or complicating agents, and the reaction shown between them in this cross is, no doubt, that which is entirely proper to them when brought into combination. Any such reaction is in all probability entirely a laboratory event,

and in nature it is highly improbable that anything of this sort would happen, owing to the rarity or absence in both of the species of this modal biotype uncomplicated by other conditions or agents productive of other characters in the gametes. No materials direct from nature have ever given any such result entirely, so that natural crosses are more complicated than the one described.

Another cross of interest is that between the modal biotype of *L. decemlineata* that was used in the first instance and *L. multitæniata* biotype *melanothorax*, with other characters those of modal standard. In this combination *decemlineata* is dominant in F_1 in all stages, especially in the juvenile, and F_1 when inbred for F_2 shows in this instance in the larvæ the same array as in the first cross, due to the same causes, and is of no further interest. In the adults, however, there come out in F_2 sharp indications of the dissociation of the gametic systems, as far as certain adult characters are concerned, showing uniformly the following types in variable proportions:

decemlineata in all respects.
decemlineata with *melanothorax* pronotum and head.
multitæniata in all respects of biotype 7.
melanothorax in all respects.
multitæniata of biotype 2 aspect.
decemlineata of biotype 2 aspect.

Of these there are some *decemlineata* that breed true; the majority are heterozygous as far as their reactions are known. The *decemlineata* with the *melanothorax* characters has always the form-index of the parent (2.699) and breeds true in part. *Multitæniata* in all respects of biotype 7 has in all tests bred true; *melanothorax* in all respects also breeds true, while none of the remainder has been found to breed true. Out of the combination I have derived thus far the following stable combinations:

decemlineata in all respects. Ratio, homozygous 3: heterozygous 8. N.=96.
decemlineata with *melanothorax* pattern; 4 out of 6 pairs mated. Ratio, homozygous 2: heterozygous 1. N.=141.
multitæniata in all respects of biotype 7; 14 out of 14 tests. Ratio, homozygous 1: heterozygous 0. N.=73.
melanothorax in all respects; 12 out of 12 tests. Ratio, 1 homozygous: 0 heterozygous. N.=134.

All of the other types that come out in F_2 do not breed true in any tests thus far made.

The point of interest in this cross is the crossing of the pronotal-pattern producing agents from one gametic complex to the other, and this is shown clearly by the finding of them in combination with the characters and form-index of each of the two parent types. Interesting also is the derivation in F_2 of *multitæniata* biotype 7 as a result of the cross, showing that the pattern present on the head and pronotum of *decemlineata* had crossed to and replaced in *melanothorax* the corresponding agents, thus synthesizing *multitæniata* biotype 7 out of the combination, and also giving *decemlineata* forms with head and pronotum of the *melanothorax* type.

None of the juvenile characters have been observed to be dissociated from their respective gametic systems, and in F_2 all homozygous forms are red where

the *decemlineata* form-index is present, yellow where that of *multitæniata* is present, and complicated when the individual is heterozygous.

The reaction in this cross is plainly of a dihybrid type in which the gametic systems represent one unit in the reaction, with all of the non-dissociated agents and characters proper to each, and the two head-pronotal patterns the other pair of characters. In reality these are the dissociated agents, although in this instance each is complex, even though acting as a unit in the reaction. I have not the requisite data to attempt a statement of the ratios present, owing to the necessity of testing out many more pairs of the heterozygous individuals, whose composition can not be approximated by inspection, and whose study is further made complicated by their great sensitiveness to conditions in the medium which act to cause extreme fluctuations that obscure their true nature. The existence of the four pure-breeding lines are, however, all of the data needed here, in that they show fully the interchange of the head pronotum-pattern producing groups as units *in toto,* and their combination with the other parental gametic system. I have only been interested in the fact of interchange and the number of possible stable combinations that are derived and the type of reaction. Crosses so plainly dihybrid in type are not worth the effort to work out the actual composition of the heterozygous types present, and especially so as there has been no indication that there was anything unusual or interesting in them.

In this cross precisely the same species bases are used, the only difference being the presence in *multitæniata* of the agents productive of the *melanothorax* type of head and pronotum coloration. The type used was not the recurrent mutant *melanothorax,* but only the transfer of the head pronotum group of pattern agents into the biotype 7 complex, displacing the corresponding complex, giving the type used. The cross had only the one difference present, that of the two pattern-agent groups mentioned and the specific non-dissociated base. In the first instance the crossing showed only uniform monohybrid reactions, the two systems remaining intact; in the second the two remained intact, but with the exception of the one interchange between the two groups of agents observed. Why this should be so can not at present be decided, but it is clear that the reaction is not the product of a chance shifting of independent agents, but is more probably the interaction of the two gametic systems upon the basis of their nature and conditions of reaction, these determining the extent and character of the dissociated and interchanged agents or groups of agents.

From this point it is possible to make all sorts of complications in the crossings of these biotypic lines, simply by adding to one or the other agent or groups of agents by the appropriate methods. Thus, for example, if to the same two stocks utilized in the preceding experiment there be added to the *L. multitæniata* line the red elytral color, the complications are greatly increased in the array of pure-breeding types found in F_2.

The red may be derived from any source, but the most convenient one that I have found is the geographic variety *variabilis.* This is readily reduced in culture to a homozygous-acting red race, and when crossed onto the combination of *multitæniata* and *melanothorax* types gives a race that is pure for all of the characters, is homozygous in action; and this, when it is crossed with the *decemlineata* race used, further complicates the F_2 array by the production of yellow, orange, and red in pure-breeding races, thus adding to the possibilities

already present to make a considerable array of possible pure-breeding F_2 extractive races. The F_1 in this combination shows nothing of interest with these lines; *decemlineata* is dominant, and the fraternity is as in the other combinations of these species, except that the elytral color is variable from yellow to red, with no observed regularity of proportions. F_2 is complicated, and I have never tried to analyze out the entire conditions present, as it required space and labor that I could not afford to devote to this relatively unimportant portion of the general project.

These experiments establish first, the point that *decemlineata* is the dominant form in crossings of these two species, and this has an important relation to some of the experiments to be presented later. It suggests also that in the testing of species in initial crosses it is in all respects best to reduce them to a modal biotypic condition, from which races all contaminating agents or characters have been removed, or the lines otherwise reduced to basal conditions in which the agents productive of diversity were eliminated. When this is done it is possible to test fully the true method of reaction between the two species and the extent to which dissociation occurs in crossing. From this as a point of departure it is possible in any instance to test by experiment the action of added gametic agents or the rôle of external conditions in the production of heterogeneity of action and product in the crossing of the two in experimental conditions or in nature.

As far as the crosses of these two species are concerned, further description at this point would add only uninteresting details and no points of general interest. Added data of the crossing of them appears from time to time in other portions of this report as it has a bearing upon the operations under discussion. At all points, however, it should be kept in mind that these two species when in combination are not in crosses dissociated to any considerable extent, and that the basal form, comprising those agents that produce the form as measured by the form-index, with which are associated many characteristic and non-dissociable characters, remains intact in the intercrossings, and only some of the more easily displaced agents are shifted. The condition in this cross is therefore quite different from that of *decemlineata* and *oblongata,* in which the reaction between the two reminds one of an extensive disruption of the two systems when they meet in the F_1 heterozygotes. The main point in presenting these details in this report is to make clear the fact that the reactions of these species in crossing are in no respect different in principle from the reactions found in many other organisms studied, as measured by the neo-Mendelian reaction in the last ten years.

LEPTINOTARSA MULTITÆNIATA × LEPTINOTARSA OBLONGATA.

The crossing of these two species presents essentially the same set of problems that are present in *multitæniata* and *decemlineata.* It is necessary that the materials be reduced to homogeneous conditions, to modal biotypic lines, before the crossing is made. When this is done and the cross is made between *multitæniata* modal biotype and *oblongata* modal biotype, the reaction is a perfectly simple one, monohybrid in all respects, without any dissociation of either gametic system. This cross is not complicated by the juvenile body-colors, which are the same, yellow, in both, and the only juvenile difference is the

pattern, which is not in experience dissociated in this combination. The chief distinction is the form-index, which permits of sharp differentiation even by inspection and separation of the F_2 extracted groups.

SUMMARY AND DISCUSSION OF THE CROSSING OF SPECIES.

It has been shown in these two chapters that organisms from nature that have the taxonomic value of species, some of which have been distinguished as such for more than a half century, can be crossed and give fertile progeny and types of reactions that are interesting. The first question that naturally arises is, are these forms true species? But what are true species?

It has been shown in Chapter II that each of the forms used here have precise habitat relations and differences, are at all stages in life distinguished by constant characters of color, pattern, form, and the many specific methods of reaction characteristic of each, between which species there are no present intergrades. Nevertheless, they cross and produce fertile progeny to greater or less degrees, and in this transgress one of the oldest criteria of distinctness. It is now certain that there is no certain line of separation in this respect, and that from complete fertility one passes by all sorts of transitions to complete infertility. What constitutes fertility or infertility remains for the future to determine. Some data of significance is gathered from these experiments.

The problem of fertility of species hybrids presents two aspects: (1) as to the factors productive of limitation of breeding in the initial cross; (2) of the factors productive of sterility or nonsterility in differing degrees in the F_1 array in breeding. In Darwin's time, and even before it was known that the sterility of hybrids between species was a complicated relation and the work of Gartner, Kolreuter, and others, as well as his own experiences, forced Darwin to admit that "we have conclusive evidence that the sterility of species crosses must be due to some principle, quite independent of natural selection," "and from the laws governing the various grades of sterility being so uniform throughout the animal and vegetable kingdoms, we may infer that the cause, whatever it may be, is the same or nearly the same in all cases." Thus Darwin clearly recognized that sterility, of which so much could be made in discussion of species and their origin and its advantage to incipient species, rested upon other principles, and in the end he concludes that "the primary cause of the sterility of crossed species is confined to differences in their sexual elements."

Sterility, in the broad use of the term, comprises several different aspects of the fact of nonproduction of progeny in a given mating. Commonest are the instances of mechanical inhibition of breeding or copulation, which is usually due to structural relations or conditions whose origin is unknown; second, there are the numerous series of instances where the gametes are able to unite and initiate developments, which, however, do not progress beyond a certain stage in ontogeny, a stage which seems in many instances to be a constant point for the particular combination; third, the array of numerous examples of the production of an F_1 hybrid, frequently with unusual personal strength and vigor, but inability to produce gametes that are capable of either fertilization or development or both. Between these three modal points there are many intermediate conditions described in the voluminous literature of hybridization.

The first group of causes of sterility is at present not open to profitable discussion, owing to the fact that in no case is it known what the agencies were that produced the mechanical inability to copulate or inhibition of the gametes to unite or even come into proximity. These are matters of existing structures, with specific modes of function, of whose method of production entire ignorance prevails. Apply interpretation as one will, nothing but a miserable array of plausible arrangements of possible causes and effects is the result. It is not even probable that these diverse inhibiting mechanical devices arose because of their utility in keeping the species pure or aided in its origin, and so we must pass them by until such time as there is real basis of discussion.

Of the two remaining categories of agencies productive of sterility and associated with the union and nature of the gametes themselves, direct experimental investigation is possible.

It has been recorded in many plants and animals that the fertilized zygotes resulting from a cross begin development, progress normally up to a certain point, then cease normal development, go on in abnormal development for a time, and then die at approximately the same point. It is needless to cite instances; these are too well known to the readers of hybridology and experimental embryology.

In the crossing of these natural species I have had many indications that the problem of sterility is entirely one, as far as the gametes are concerned, of their factorial composition and of the position and action of these agents in the gametic complex. *A priori* this might be asserted, but can experimental confirmation of it be obtained, investigated, and the rôle of specific agents determined as to their relation to the production of this phenomenon?

Uniformly in all of the species used the stocks fresh from nature do not cross with the same ease or certainty as they do after they have been in the laboratory under the same conditions for several generations. This has been noted in one way or another for nearly a century, and Darwin makes much of it in his writings, but I do not know that anyone has made a serious investigation thereof. With the species used I have had no difficulty in their breeding under the cultural conditions provided for them, so that my problems are not those so often mentioned in the breeding of wild organisms—of their refusal to breed under domestication. I have not found any that would not reproduce when given the proper food and conditions in the medium. Nevertheless, while I have had constant success in the breeding of wild species in my laboratory conditions, the crossing of these shows in all most decidedly that at the start the cross is not only far more difficult to make, copulation is not so easily effected, and after copulation, if fertilization is effected, the mortality in the developmental stages is immensely greater than in the same type of cross in the same strain two or three years later. Data upon this point is given in condensed form in table 22 which shows in the three species *L. signaticollis, diversa,* and *undecimlineata* the change in respect to the fertility of the matings in crossing of these three during the first six generations of captivity. In the pure species cultures there has been no appreciable change in the fertility of the species from the introduction to the end of the sixth generation, and the matings not producing adult progeny are the same in the first as in the last generation; nevertheless, in the crosses there is in the matings made in the stocks fresh from nature much lower

fertility than is found in later matings, although at no time does the fertility become equal to that in pure stock matings in any of the species.

In any combination between these three species there is never entire infertility in any line found thus far in nature. I have recorded in table 22 only matings in which copulation was observed or known to have taken place, but in these matings there are instances in which there is no copulation of the pair and they pay no attention to one another. These cases have their causes in the breeding reactions of the parent species and are in part instinctive reactions dependent

TABLE 22.

Condition of stocks with reference to time under cultural conditions.	Species crossed.	No. of matings.	Copulated No. of progeny.	No. giving eggs, fertilized that died.	No. of whose progeny died as larva on hatching.	No. of whose progeny died as larvæ.	No. giving adults in F_1.	No. tested in F_1 and found fertile.	Infertile or No. of copulation.
Direct from nature ..	L. signaticollis × L. diversa.	132	51	19	7	5	50	43	0
Second to fourth generations in conditions of culture.	.....	185	46	11	3	1	124	77	0
Sixth and later generations in culture.	.....	151	18	4	3	2	124	67	0
Direct from nature ..	L. signaticollis × L. undecimlineata.	211	65	41	17	19	69	60	0
Second to fourth generations in conditions of culture.	.....	179	31	23	9	4	112	97	0
Sixth and later generations in culture.	.....	243	19	5	7	7	205	174	0
Direct from nature ..	L. diversa × L. undecimlineata.	73	19	3	4	6	41	38	0
Second to fourth generations in conditions of culture.	.....	151	31	5	8	2	105	78	0
Sixth and later generations in culture.	.....	82	7	1	0	1	73	60	0

upon sex-attractions of one kind or another, most commonly that of odoriferous secretions and possibly to other agencies.

I have been most successful in the crossing of these species if they are mated at the height of the first reproductive activity, at which time the instinctive reaction against an outcross seems easiest to overcome, aided perhaps by the pressure of accumulated reproductive elements within. My data show that in these species there is at the start infertility such that only a small proportion of the matings give adult progeny and that continued existence under the same conditions results in the raising of this fertility to a considerably higher percentage in the matings, but never entirely reaches the fertility of the matings within any of the species.

In Chapter IV, I have shown that the rate of development, the determiner, *Ac*, and its values had a rôle in the production of certain interesting results in the crossing of these species, and examination of the records of different matings gives data which show that the same agent is in some respects concerned in the production of the degree of sterility in these crosses. The data given in table 22 are drawn at random from the records of the crossing of the different species and the stock matings, under the same set of cultural conditions, and in that time the species attain the same *Ac* values, about 60. I find that in strains of *signaticollis* in which the *Ac* value is artificially kept at about 40, that the degree of fertility in crosses is lower and approximates the initial condition on being taken direct from nature.

Added data in the same line comes from the testing of one of the races that arose from the crossing of *signaticollis* and *undecimlineata* described in Chapter IV. This curious *undecimlineata* race arose as a pure-breeding one in F_1 and was characterized by its excessively slow ontogeny and long life-cycle, irrespective of conditions in the medium. This race I crossed repeatedly with *signaticollis* having the *Ac* values between 40 and 60 as a test reaction to break it up and so determine its composition, in which the crossing was successful in 11 out of 131 trials, and then only with the *Ac* value at 60, never at 40, but even at *Ac* 60 the cross is sterile.

From these data, gathered by the wayside as the by-products of other experiments, it is shown that the activities whose presence and action is symbolized by *Ac* and its value expressed in elapsed time is an agent in the gametic complex whose action is productive of changes in the degree of fertility in the crossing of these species. The data accumulated show that when the values of *Ac* are wide apart fertility is diminished, when equal fertility is increased, but that the attainment of the same values in the *Ac* determiner does not raise the fertility to the standard found in the pure species.

This experience is in line with the collective experience of those who have spent years in crossing organisms as was well stated by Darwin, Gartner, Kolreuter, and others among the older students of these problems; but why do the species from nature become increasingly fertile after they have lived under the same conditions of existence for varying lengths of time, and what is it in the species that changes?

In the species *L. signaticollis, diversa,* and *undecimlineata* it is the *Ac* determiner values that change, made manifest to us by the rate of ontogenetic development under the standard conditions of the experiment, although not the least evidence is to be had as to what changes in the physical composition of the gametes. Numerous possible causes can be suggested, but evidence is wanting that it is any of them. It would be interesting to know how general the relation between this changed rate of development and the degree of fertility is, but I have not been able to find any data in the literature that has any direct bearing upon the problem.

Even though the rates of development be the same as can be made in experiment, the fertility never reaches the point found in the normal cross, so that there are other factors in addition to the *Ac* determiner that are not altered, or not enough, by the changed conditions of life so that fertility reaches the normal for the species. A point of interest in this connection is the interfertility of extracted F_2 types, such as are derived from the crossings of these species. In

the extracted types coming from the crossing of *signaticollis* and *diversa,* the degree of fertility is as high or higher than in either of the parent species. Comparison of the data shows clearly that there is relatively higher fertility in the F_2 extractives, showing that there has been a change in the nature of the gametes by the reaction of crossing, and inhibition of uniform fertilization and development is removed by passage through the F_1 reactions. A point of interest arises in this connection, namely, the increased interfertility between these extracted F_2 stocks and the parent strains.

The reactions in these crosses are of monohybrid or trihybrid types, and the extracted products show no change in any detectable attributes or qualities, so that the reason why the extracted products of crossing have higher interfertility is a problem for further study. There clearly is some change in the gametes as a result of the passage through the F_1 reactions which in some way removes the barriers to complete fertility in the matings of these otherwise distinct types.

I was further interested to see what the data would show with respect to the fertility of the F_2 extractives coming from different crosses. I had compiled from my mating-records data of the crossing of F_2 homozygous-acting extractives, and although the crosses of this sort have not been numerous, the data derived as the by-product of other experiments show clearly that the fertility is strikingly lower than in the matings of F_2 extractives from the same type of species cross.

Out of these data three interesting general points come: (1) the demonstration that alternation in the degree of fertility follows directly any change in the value of the *Ac* determiner; (2) the demonstration that F_2 extractives have entire interfertility between themselves and with the parent species; (3) the F_2 extractives from different species crosses, but of the same extracted type, do not have the same interfertility as extractives from the same type of cross.

In the crossing of the species *L. decemlineata, oblongata,* and *multitæniata* the *Ac* determiner values are nearly the same and culture of these species affects no appreciable change in the *Ac* value; nevertheless, the fertility at the onset of any stock direct from nature is decidedly lower than it is later in its history.

As in the first three species, the fertility between these three never has reached in experience the same ratio of progeny production from the total matings as it does in the normal stocks under cultivation, but the F_2 and F_3 extractives show precisely the same relation that was found in the first group of species, showing that the principles at work are the same.

The fertility between these two groups of three species is very low, and thus far the only series of crosses that has been at all successful are the two lines from the crossing of *decemlineata* and *diversa,* described in the early portion of this chapter, and which gave such unusual results. Only two have given adult progeny out of many trials. No other successful crossing between the two groups has been attained. This has been attempted, but there seems an aversion of the two series to one another, so that copulation can not be obtained excepting at the climax of reproductive activity, and then is best attained if the antennæ are cut off or covered with neutral gum, so that the sex-reactions depending upon the odors of the species secretions comes feebly or not at all into play. By subterfuges of one sort or another I have been able to make all the possible combinations of these two groups of species, able to obtain matings and fertilized

eggs in all of them, most of which developed for short periods, but few of which hatched. Very many die before or at the period of the forming of the limb-buds. The two groups have quite different rates of development, *Ac* values, and differences in these may be primarily responsible for the death of the embryos.

It may be possible to produce hybrids between these groups by the altering of the *Ac* values through the use of external conditions, but thus far the permanent alteration of this character has not been affected by this agency. It is shown in this and the preceding chapters that this character or the agents which it represents are specific for the species, and, while capable of change, has in all thus far tested shown rigid upper and lower limits not transgressed by the action of the pressure of conditions in the medium.

In one way or another the gametes of the different species in this genus are able to unite and begin development, some to complete it. The problem of the intercrossing of species consists, therefore, of two sets of problems: the initiation of the mating reactions, which are independent of the second, namely, the interactions of the gametic systems when placed in the zygote. Evidence is derived from these experiments showing that in the gametes the production of F_1 adults is directly associated with rates of development in the two gametic systems, and that likeness of rates is productive of fertility and development, unlikeness of infertility and cessation of development; but there are other at present undetermined agents present contributing to the infertility and non-development. Many interesting and suggestive openings into this most difficult and important portion of the problems of evolution have been obtained, and I hope to be able to follow them further.

Suggestive points with regard to the inhibition of the reproductive reactions have also arisen in the course of this crossing, which collectively indicate—in some instances show conclusively—that the reproductive isolation of these species or types is independent of the factors governing the interaction of the gametes and in most instances is an incident in the origination of the line, a by-product of the process through which the form was produced, and I have observed, in the course of this work, instances of this isolation which have arisen in experiment by the reaction of origination, and the isolation has no relation to utility, purpose, or adaptation, nor plays any rôle in the origination of the line. It is a purely physical resultant of the reactions that have taken place in the production of the specific form. In the data of crossing species already given there is positive evidence for this conclusion, while later portions of this report contain added data in support of the same proposition. An interesting instance of the rise of a race that is physiologically isolated in different ways is the line that arose from the crossing of *L. diversa* and *L. decemlineata.* This race or species crossed with *L. decemlineata* is slightly fertile, and entirely infertile with *L. diversa, L. signaticollis* and *L. decemlineata,* and no method has been found that decreased the infertility in any degree. This was true of the line so long as it was maintained and was the direct and completed product of reaction that gave rise to this race, so that there were produced factorial conditions that effectually inhibited the reproductive activities with the nearest of kin, the parent species; nor has fertility with other species changed as far as has been tested. The condition is not that of either parent, but something new, a fact that is clearly manifested by the infertility with both parents. *A stock of this character arises as a*

group, not as an individual, and its isolation is a process of production and not accumulated progressively in any manner, from any process of survival, selection, or utility. It is purely a result of the reaction, and there is no reason why it should be present other than that the gametic factors active in the production of this line in their interaction produced a stable complex such that this was one of the resultant products.

The stock is also of interest in that it shows that other aspects of the isolation problem are touched upon in an interesting way. The resultant combination of different characteristics of the parent species has given habitudinal isolations that are barriers to the breeding of the form in one direction at least. Its food demands are for *Solanum chrisotrichum,* or *S. hertwigii,* upon which it feeds, and upon which the only form with which it has been made to cross does not feed, and there were other differences of habits and developmental rates, and on the whole it was in many ways thoroughly isolated from its progenitors. All of its character associations are the product of the act of origination, are in no respect due to slow development or survival, and are entirely produced without regard to utility in the form. Conditions of isolation seem to act in this useful way only after they are fully established in the special life-relations into which the processes of production have by chance happened to place it. It is not improbable that the same product under other conditions of life might well have the same attributes and qualities in all respects, which might not there serve at all in the capacities to give the degree of physiological isolation that the observed series showed.

What constitutes fertility or infertility is clearly and only the capacity for interaction of the gametes and inhibition of the parents from breeding, both of which are the direct product of factorial composition, method, and rate of reaction of the combining gametes. Whatever there may be of inhibition in reproduction is, in some instances at least, solely the indifferent results of the process of origination; but how generally this is true of the conditions existing in nature is and must remain entirely a matter of opinion and interpretation.

This is not the only instance in these experiments in which the problems of fertility in species crosses has been elucidated by data derived as a by-product of other work. In the crossing of *undecimlineata* and *signaticollis,* as described in Chapter IV, there arose under certain conditions a race that was exceedingly slow in its developmental reactions and equally long drawn out in its life-cycle. This race was produced by the single series of interactions of the cross as a group, with all of its attributes and qualities present and formed by the reactions preceding its appearance in an adult F_1 array. It was, as shown by the test, of a heterozygous nature and could by proper means be broken up, but in the usual conditions of life it was as stable as the majority of species in nature. It would breed, as far as the act of copulation was concerned, with either of the parent species, but the different rates of development, or something that was the product or associate of this difference, produced a huge mortality in the progeny of these crosses. In this respect it is isolated, and while it might go through the reactions of breeding, the differences in rate of reaction in the gametes, or factors associated therewith, directly eliminated the probability of more than sporadic or small numbers of progeny.

This race had another interesting aspect of its composition which in the conditions of culture was of no moment, but in nature would have been in the form

of an added agent, isolating it from its relatives in breeding. It was the uniform experience with this stock that it came out of æstivation 1 to 3 weeks earlier than either of the parents under the same conditions. This was uniformly true where all had gone into æstivation in the early winter months voluntarily; under identity of conditions this special race was out first and well started in breeding before the parent lines had started into action. Had this been the reaction in nature, and I can discover no reason why it would not, the new race would then be out and breeding in the spring before the others had emerged. Trivial conditions of this sort in the composition of organisms are productive of not a little of the separation of natural groups of organisms, and in the main it seems most probable that characters of this sort have arisen as the result of the product of the reactions productive of the origination of the group rather than in the indirect ways of survivals and slow accentuations through long periods.

In these experiments I have in the main eliminated, by the method of working, the sterility that comes through nonbreeding as the result of new conditions by the provision of conditions such that the stocks from nature could breed in my laboratory, and although there is much in the effects of these conditions of life in their action upon the reproductive activities, I have eliminated as much of it as possible, my operations being adequately complicated without the addition of this group of factors. There is abundance of evidence that this relation to the conditions of the medium are vastly important and govern the breeding activities of all organisms directly in nature and in experiment.

Probably the most common record in the accounts of the crossing of species is that of the sterility of the F_1 progeny and the inability to obtain F_2 progeny of crossings back upon either parent form. It has been discussed adequately in the literature; tests by breeding and examinations of the germ-glands have shown that there are slight or extensive derangements of the gametes, and there the entire matter rests. In my experiments I have had but few instances in which if F_1 progeny were produced they were also not able to produce F_2 and subsequent generations. It was found in the peculiar race of *undecimlineata* mentioned in preceding pages that often, when it had given progeny in crosses, it gave no second generation when these F_1 were inbred. No reason could be discovered except that often the males or less often the females would not develop any mature germ-cells. Examinations showed that the gonads were not active and were producing only a few or only defective germ-cells. In normal materials similar individuals are also found which have a relation to lines of sterility in the race. In these hybrids the reactions were the same and the defective nature of the gonads much like the conditions in sterile individuals of normal stocks. There the matter rests for the present. I have no means of determining what the sterility is due to, whether to disease, inheritance, or incident agents, but these cases are sterile independent of any conditions in the medium that I have been able to discover.

It is a constant experience in the crossing of these species by close attention to the conditions of life, as temperature and food relations, that in the production of F_2 there are effected many times in such production a distinct increase in the fertility, and often in practice the obtaining of F_2 has depended upon these niceties of adjustment in the conditions surrounding the organisms. One is led to wonder to what extent the recorded sterility of F_1 hybrids among animals, especially in the crude conditions of zoological gardens, may not in reality be

due to this cause rather than to the defective reproductive apparatus. Nor is the fact that the gonads on examination have not produced gametes proof of their sterility, because I have seen examples where there was not the least activity until I had made some trivial change in the environmental complex. The reproductive process is so delicately balanced in the organism, so tied with diverse factors within and without, that the problem of sterility presents endless complications to the experimenter.

In this investigation fertility and sterility have been only incidentally studied in the practical aspects of the breeding operations; nevertheless, as a by-product from these routine crossings of species, data are derived showing that the problems of this character are entirely matters of factorial causation and that the production of conditions of fertility or intersterility are the direct result of the reactions incident to the origination of the stable type, which, if it were found in nature and first seen by the taxonomist, would have been a species. Isolation in reproduction and intersterility have long been and still are by many considered the distinguishing mark of species, but I can not see any *a priori* reason why this is necessarily so. Specific distinctness in the non-living is solely the possession, by the contrasted materials, of constant and characteristic attributes and qualities, manifested to our perceptions, such that they may by us be separated one from the other. They may be specifically distinct, yet interact with violence, ease, or not at all. The capacity for interaction is not a criterion of specific distinctness. In organisms fertility or sterility in its many manifestations is only the capacity of the specific masses to react, and the mere fact that they act readily and uniformly when combined, with difficulty, or not at all, has no bearing upon their specific distinctions, which in each are proper thereto and constant at all times, irrespective of the capacity for interactions. Enough has been presented at this point to show the trend of ideas with reference to these problems and the substantial support that comes to them from these experiments in the crossing of species. Interspecific sterility in animals has been regarded as an indelible characteristic of specific distinctness; but as Darwin was forced to admit, and all since have seen, this was not universally true. Darwin, however, thought that it was true in nature, but could be removed by domestication. Cultural conditions can alter the factors concerned in the production of sterility, within the limits of fluctuation proper to the species, not beyond, and further change must come as the result of the product of the reactions of interacting gametic systems, wherein the factorial nature of the system is itself changed. I have reared *diversa* and *decemlineata* for many generations and seasons in the same conditions, but the degree of fertility is no higher now than at the beginning. Others have changed within the limits of fluctuation of some specific and efficient factor, not beyond, and perfect fertility is not in experience produced in any instance by the conditions of culture.

THE PROBLEMS OF THE NATURE OF SPECIES CROSSES.

De Vries has clearly crystallized the general opinion so long prevalent that the crosses of species are different from the crosses of varieties in principle, in his now familiar statement that there are two main types of crosses—the bisexual or Mendelian and the unisexual—in which the chief criterion is that the bisexual gives in F_2 segregation into different groups with interchanges of the characteristics of the parents, some of which breed true as varieties, while the unisexual do

not segregate, but are fixed from the start. This experience is projected backwards from that point to a hypothetical difference in the gametes, which is not put to test in the effort to discover whether it is capable of dissociation and thus determine its nature. Uniformity of action in succeeding generations has been the test of the constitution of organisms in the past; but, as is increasingly shown by the work of the Mendelian hybridologists this is not true, and in this work the crossing of species has shown us both of the main sorts of crossing reactions that De Vries and the older authors distinguished in the work of hybridizing organisms.

In the course of this investigation I have had under cultivation and crossed many species from nature, and as the collective result of this experience, some of which is given here, I must conclude that there are not two main kinds of types of crosses, but that in the crossing of organisms there is evidence of only one type of reaction, namely, that the union of the gametes in fertilization results in the interaction of the two gametic systems in F_1, in such fashion as their structures and compositions permit, to produce the resultant adult zygotes, and which in gametogenesis produce, through the action of the two intermixed gametic systems, separations of these with or without dissociation and interchange of larger or smaller associated groups of gametic factors.

Examination of the data presented in the description of the different crosses shows, as for example in the simplest series, the crossing of *diversa* and *signaticollis,* that from the same parent stocks different types of reaction were obtained; sometimes the reaction was such that it would be described as the bisexual, at others it was only the unisexual, and at still other times it was intermediate. The parents are quite distinct, do not live in like habitats, and are not completely fertile, but their interfertility is easily altered within limits. It is shown in these experiments to what the mechanism of this difference in the reactions of crossing are due and how they may be produced in experiment. It is further shown that the pure-breeding lines that arise in F_1 are capable of being in some instances broken up and their composition determined, showing that they were in actual composition heterozygous, but through the action of the gametic agents the relation of the factors in the gametes was so altered that it persisted, giving the appearance of a pure race, because its successive generations were alike when bred on without test. Proper conditions readily dissociated it, showing its heterozygous nature, which was also strongly indicated by the trimodal polygons of the fraternity. In species crosses in which the two or more types of reaction are observed, if when analyzed show that the principle of reaction is the same in all aspects thereof, we may well speculate as to the probable nature of many untested series in which the only test applied was that of their " breeding true." The point has been reached where the only indication that " breeding true " gives is that it tells little of the composition of the line that breeds true, and as a result I have distinguished in this work very carefully between *homozygous in gametic constitution,* and *homozygous in actions.* The two may or may not be present in the same line.

In these experiments many lines arise that are heterozygous in composition, but have been homozygous in action, and it has been shown that some of these can be proven to be such by the proper tests, either complete or in part, while a few lines have with present methods and knowledge resisted the effects to dissociate them in tests. These lines, which have resisted the efforts to dissoci-

ate them, are of the nature of what has been called fixed hybrids, numerous examples of which have been described in the literature, especially that of horticulture and in the older writings. There would be little question as to the stability of these types and of their position in nature, and no doubt they would receive at the hands of taxonomists the designation of species. However, it is shown in the examples that have come up in the course of these experiments that they are heterozygotes, of such arrangement of the factorial agents that the separation of the two gametic systems in F_1 gametogenesis is either obscured as in the fixed heterozygous lines arising from the crossing of *signaticollis, diversa,* and *undecimlineata,* or suppressed, as in the products of the cross of *diversa* and *decemlineata.* As far as the evidence shows us this diversity is purely a result of the factorial action, within and without, that is productive of these conditions in the crossing of species.

An interesting series of data are derived from these species crosses, showing that the Mendelian reaction, which is so universally present in them all, is a type of reaction that is entirely independent of the size of the factors that are transposed between the two crossed species. It is interesting to find, as in the cross of *diversa* and *signaticollis,* when the *Ac* values are similar, that the reaction in crossing is in all respects most accurately and typically Mendelian in its showing of F_1 and F_2 arrays; but more of interest is the action of the entire gametic complex of each of the parent species as a unit in the operations, giving the most perfect monohybrid displays. There is no dissociation from the masses; each in all experiments retains its integrity; and the resultant reaction is of the purest monohybrid type possible, and complications come in only when the parent stocks differ in the *Ac* values, which differences produce not dissociations but derangements in the nature of the F_1 heterozygotes, giving arrays that might easily lead to false conclusions, as the nature of the gametic constitution of the parent stocks.

In more complicated crossings, as in that between *signaticollis* and *undecimlineata,* the results show uniformly the retention of the integrity in the main of each of the gametic systems, but with some dissociation of certain juvenile characters of color and pattern that are transposed in F_1 gametogenesis, giving the expected chance array of the F_2 combinations of different degrees of stability, precisely following theoretical expectation on the basis of the trihybrid Mendelian reaction. Also, there arise the same general type of complications from differences in the *Ac* values of this cross. Between this cross and the preceding certain important differences can not fail to be noted.

These two crosses *show that the Mendelian reaction in type and in operation is independent of the character of the substances involved,* and this is shown clearly by the action in the first cross of the total gametic system as a unit, and in the second by the action of the larger portion of each of the two systems as units and the simultaneous action of two portions of them also as units. These two dissociated portions—the color-determiners and the group of agents productive of the larval pattern—are not of the same nature or extent. One is probably a single agent, not capable of present change or dissociation into smaller agents, and this is the condition of the yellow-color-determiner that is dissociated and recombined. The larval pattern is known to be a system of minor agents that collectively act to produce the pattern, a conclusion that is warranted by the capacity shown for alteration of this pattern and the removal of specific portions

thereof, the presence of which is dominant to its absence in typical Mendelian reactions. In this species cross the typically Mendelian reaction, trihybrid, is entirely a product of the number of the dissociated groups, and independent of the character or size thereof.

In the crosses between the species *decemlineata, oblongata,* and *multitæniata,* another aspect of the same problem appears, namely, the complexity or simplicity of the reaction depending upon the complexity of the gametic constitution, as in the cross of *decemlineata* × *multitæniata,* where factors added to the complex also added complexity to the type of reaction resulting. The cross of *decemlineata* and *oblongata,* however, showed still other conditions in the extensive dissociation that followed the combination of these two in a hybrid reaction. In *decemlineata* × *multitæniata* the modal biotypic conditions in homozygous lines reacted in strict monohybrid reactions; added factors to either disrupted this and also induced dissociation in the gametic complexes; and so through the entire series it is uniformly the result that the reaction which follows the combination of these species gametic systems is the direct product of their differences, and the dissociation resulting depends upon the number of equivalent factorial groups capable of metathesis. This sequence of events following the cross we may call the *Mendelian reaction* and not Mendelian heredity.

The chief point that I desire to make in this connection is that in the crossing of species direct from nature there is but one fundamental type of action, which depends entirely on the factorial constitution of the gametes combined, multiplied by the conditions of the medium at time of combination, and that the resultant reaction is the direct product of their differences and the capacity for metatheses between equivalent factorial groups. If the reaction proceeds under conditions in the medium that are common to and act in like manner upon the two systems, the reaction and products will appear in proportions and combinations that are the product of chance, but if the conditions of the medium act to accelerate action in one gametic system or to retard it more or less than in the other, then the dissociation and combinations are disturbed and the resultant array of products is entirely dependent upon the nature and action of this active agent in the medium. This Mendelian reaction is our first and at present most important means of investigating the gametic constitution and action of organisms. It is not a law of heredity; it tells us nothing of the way characters are maintained through countless generations of similar organisms nor why characters exist at all; but it shows a first method of investigating the factorial agents in the gametes that are, in ways at present unknown, concerned in the production of the characters in endless repetitions.

But once for all let us discard the notion of characters and of kindred conceptions, and recognize that the presence of a character in an organism is there as in the inorganic, the end-result of the interactions of the component non-living substances, and so the physical basis of these characters that are used here as mere indicators of the presence and type of reaction present may be only HO groups in the material substance that bridges the gap between generations. We do not know the exact agents that produce these end-results, but their precision of action, and the certainty of their duplication, times without number, in the same line of living beings can admit of no other viewpoint than that the production of a character is, in organisms, the same resultant of the physical interactions of the materials of composition, and due to the same precision of process that pro-

duces blue rhomboidal crystals when $CuSO_4$ and $5H_2O$ are combined. Not that it is the same, but that it has the same physical principles of operation and causes of the resultant characters, which are after all only temporary states of stability in the ceaseless integrations and dissipations of matter that characterize the progression of organisms in nature.

In this crossing of species from nature I have presented for the most part only data that tested to the limit of present operations the truth and application of the Mendelian reaction. I have found no blends, no instances, totally at variance with the principles of action of this test of gametic composition, and while it is true that there remain in this account lines that have arisen whose entire composition has not been entirely solved, or whose production is not entirely clear, still no instance has been found that has not at least given exact evidence that the Mendelian reaction and the principle of gametic factorial agents are not present and operating, even though at present obscured by unsolved portions of the problem. This latter is, however, open to continued test of experiment, and empirically it is possible to progress with certainty in the solution of these complications.

The literature is abundantly supplied with instances of the rise of pure-breeding and stable races as the product of an initial-species cross, and in them the fact of their pure breeding in following generations has been the criterion of purity, blending, and kindred assertions concerning them. Many Mendelian workers, Bateson especially, has expressed doubt of this being true, and while it may be true in some instances, I am at present not informed of any instances where the testing of them has been even a tenth part as rigorous as that given in similar lines in my experiment; and so, on the basis of experience, in this series we may well ask that there be other tests applied than those of " breeding true " and of the simpler routine Mendelian reactions.

In my experience this Mendelian reaction has thus far stood the test of the most hostile treatment I could give it, and everywhere it has proved to be certain and precise, correct, and when one dissociates it from misconceptions and the drag of biological units it takes on a new significance and in investigation becomes one of the most important tools of progress that we possess to-day. The probable nature of this reaction is discussed in Chapter IV.

THE PROBLEMS OF THE CROSSING OF SPECIES AND SPECIES PRODUCTION.

In spite of assertion that the problems of evolution are those of character origin, it must still be admitted that the problem of the origin of the groups in nature that are called species is a real problem for investigation. The older writers upon this problem have interpreted and argued the situation over and over again, with the well-known results that the problem of the origin of these groups is by no means solved, nor is the probable mechanism of production much more than a matter of opinion.

Considerations of origins have largely centered about single individuals as the progenitor of the group, or at least a pair, in the thoughts of those who conceived of species rise through saltations, while the separation or differentiation of a group by slow changes remains largely a pure speculation. It is not at present possible to decide how any given species in nature arose, nor how long it has existed, aside from the evidences derived from fossil remains, which are on the whole fragmentary and insecure. All that is possible is to discover

methods by which groups might originate in nature, and if possible detect or produce such origins in experiment in nature.

The idea of the rôle of crossing in the production of natural groups in a state of nature as an agency of any importance is of recent origin. The older writers admitted the possibility of crossing in nature, but, following Darwin, thought of it as incidental to other operations, often as the product of a destruction of the barriers of interspecific sterility, while in the minds of all the main type of production has been that which was the product of dichotomy of some ancestral line, either by slow or rapid processes of separation. This conception is retained in De Vries's theory of species production, the separation being one of rapidity instead of by slow transformation. Since the rediscovery of the *Mendelian reaction* and its verification in numerous instances, the experiences and expressions of opinion have been increasingly towards the idea that it may be possible that species in nature are of hybrid origin. One of the first of the modern writers to express this opinion was Morgan, and it has been raised also by others, while the findings of many observers have shown that crossing is going on in nature between natural groups, but to what extent no one has any information.

The great problem in all species origins has been that of the rise of the initial group, and the discussions have to considerable extent centered around the fate of the first ancestor and what was necessary for this lone individual or pair to encounter or overcome in order to survive and produce progeny in the location. It seems to me that some of the results of these experiments in crossing species give what may be a method of the origin of groups in nature which under the conditions of existence would breed true without limit and which might rarely throw a sport as the result of its hybrid nature and be in all respects a true-breeding species. Any one of a number of the pure-breeding fixed heterozygous lines that have appeared in my cultures would have survived in nature and gone on as effective members of the fauna of the location in which they arose. This appears as a possible method of origin; I do not have any data as to how probable it is, nor do I know of any method of deciding whether species in nature have or have not arisen by this or any other method. This type of origination seems logical and suggests that it is of common occurrence in the production of natural specific groups.

Precisely the same general type of sequence of events is productive of much of the specific diversity in the nonliving series in nature; the specific thing, compound, mineral, or complex rock is the immediate product of the reactions of the interacting combining materials or factors of composition, and the product appears fully developed and in amount corresponding to magnitude of the operations. If organisms are complex compounds whose operations are purely physical in nature, I see no *a priori* reason why in the origination of specific organisms the process should not be in the main of the same order, and its rise follow, as a group of varying size, entirely dependent upon the magnitude of the origination process. In this way it is possible to conceive of and possibly produce experimentally the rise of groups in nature by processes akin to those I have witnessed in the laboratory, giving pure-breeding, distinctive, physiologically isolated groups. The conception and experience is interesting and worthy of extended test in nature and in laboratory experiment. That it is not an idle one is fully shown by the products derived in the experiments described in the preceding chapters.

I have given data in the preceding pages to show that these species, of which I have made use in my experiments, are in no respects special cases in any way, but that in the principles of reaction in crossing they show the same phenomena that De Vries so clearly puts into two categories, which without extensive testing appear to justify this distinction; but in the examples that I have had to test there appears no true distinction. Breeding true is not a test, and instances that have been classed as unisexual must, I think, be adequately tested before it be admitted that they show a special type of reaction. Throughout, the crossing of species is fully in accord with the principles of factorial composition and action, and in this the *Mendelian reaction* appears most prominent as a method of analysis.

CHAPTER VI.

THE PROBLEMS OF HETEROGENEITY.

INTRODUCTION.

In Chapter I it was shown that the characters of organisms are grouped under three chief categories: the *specific properties* belonging to the specific living kind, which can not be altered without change in the identity of the kind; *attributes,* belonging to and distinguishing bodies of the same kind from one another; and *conditions,* or states of being or activity which can be changed or removed without altering the identity of the body or its kind in any way.

Since organisms have specific properties, attributes, and conditions, and because evolution is entirely dependent upon change in the specific properties through transmutation and secondary adjustments in the attributes and conditions, it is important to understand what transmutation signifies and to discover as many of its modes of manifestation as possible before attempting to produce new states or to recombine existing states into new kinds of living substance.

It is essential that by *transmutation* should be understood only those differences giving permanent change in the specific properties. Many of the differences found in any population—"variations" in the older terminology, and which may give permanent changes which may be factors in evolution, are produced by *metathesis* through the rearrangements of the factors productive of organic characteristics by the interbreeding of two more or less unlike types. In this reaction, while differences in bodies and in substance may result, it is not transmutation but heterogenetic rearrangement, which may or may not be accompanied by transmutation. "Variation" in the older usage is synonymous with the two divisions recognized here—*i. e.,* the transmutation of qualities and recombinations of existing characters into new complexes. The first is *transmutative heterogenesis,* or the origin of new factors, the latter *metathetic heterogenesis* or the rearrangement of existing factors.

In nature, these two phenomena are continuously associated and active in the production of the diversity of specific natural forms. It is necessary to untangle the relations and interactions of these two methods of producing diversity before proceeding with the problems of experimental evolution. All too often "variation" has been thought of as a process; it is in reality devoid of unity of cause and presents unity only in the diversity of end-results, a fact most clearly stated by De Vries.

If further progress is to be made in evolution investigations, it must be known what the conditions of organisms in nature are, and these must, as far as possible, be analyzed and determined by the methods of modern experimental analysis in the laboratory. The latter will give us a glimpse of what may happen in the population observed, and will give accurate knowledge of the way recom-

binations are made and how transmutations may arise. The census-taker and field naturalist will see one side of the problem, the laboratory student another; both are right in their frequently hostile utterances, but both partly wrong.

In the making of a census in any population these main facts are desired: the number showing a particular character; the amount and permanency of the characteristic shown; the minimum, maximum and mean deviation presented; the direction and, if possible, the cause of the condition.

An entirely false conception of the nature and rôle of small fluctuations has arisen through the uncritical use of biometric methods, and while it is undoubtedly true that many characters, as for example the variation in any meristic series, do follow the law of probable error in their measured dimensions, is it certain that the differences in number or dimension are the essential features of the heterogeneity? These numerical differences, after all, are but the result of operations not discoverable or measured by the methods of the census-taker. Moreover, in color-patterns in animals or plants the amount of the color present may be measured in terms of the area exposed, and from this data the biometrician then proceeds to an exhaustive mathematical analysis in which the result, if any at all, is mathematical and not biological.

The biological facts of the arrangement of the character in the organism, the relation of this to surrounding conditions, and its effects as it varies upon the welfare of the individual, are points which may be of paramount importance; but with these the statistical method does not and can not deal.

PROBLEMS OF HETEROGENEITY.

In the literature on "variation" confusion reigns supreme—dogmatic definitions, anticipatory assertions, arguments without point; it is small fluctuations with preservations per utility and described *à la* biometrics, or it is discontinuity *per saltum* preserved by Mendelian recessiveness or dominance, or it is determinate variation ever in line conditioned by organic growth or by orthogenesis repeating ancestral states and adding new ones, which are held in line by internal automatic regulating and determining tendencies; or again, it is an inherent tendency to vary determinately, indeterminately, or *per saltum*, and so on *ad infinitum*. In reality there are many processes that are involved in this confusing mass of phenomena which are called variation.

In any population two general sets of operations are interacting. There is the action of the total population as it encounters the conditions of existence, the way in which it responds to its environment and in divers ways meets incident physical conditions and reacts to social relations of the society of which it is a member by population responses. Opposed to this is individual action tending to diversity, frequently to extremes. Both are but parts of the same process; only in the mass action of the population there is obtained a view of what the final effect of the innumerable processes which went on among the individuals in the production of the generation is upon the population in each generation. From the individual is obtained an idea of the methods of interaction, but this could never give complete knowledge, even in a limited and simple population. So, therefore, for the present at least, the general action of the population must be considered until it is known more completely what the mass effects may or may not accomplish in organic evolution.

Darwin, in the *Origin of Species,* conceived of heterogeneity in a far less refined manner than that current now. Recognizing that individual differences were numerous and in many instances showed intergradations, but that spontaneous departure or sports were also found, he expressed his inability to draw a hard-and-fast line between them. He further saw clearly that the nature of the organism and the nature of the conditions were always concerned in the production and determination of the directions of variation, and the effects of these essential factors in the cause of variation were clearly to produce two kinds of "variation" response, definite and indefinite. "Definite variation" is variation in which all, or nearly all, of the individuals in a generation living under the same conditions of existence varied in the same way or direction, and "indefinite variation" was the condition where the individuals did not vary in the same, but in many directions. Definite and indefinite variations are clearly always mass action, and continued definite variation may result in decided change of the population in one direction, while indefinite variation might equally well result in change, but there would be diversity in the population. Darwin applied this conception of the response in heterogeneity to the departure of a single character or a group of characters; to the individuals of a local group or to a species as a whole. I believe that this recognition of the method of response in a population is too much neglected and that it is of vital importance in evolution in nature. Darwin's exposition of it leaves neither possibility for misunderstanding its character nor reason for not testing its importance as a transmutative factor in nature.

Since Darwin, the same two words are often used to designate two entirely different conceptions with respect to variation. Many of the neo-Darwinians, the Lamarckians, the followers of Eimer, and especially workers in the lines of paleontology, comparative anatomy, and descriptive ontogeny, have used definite and determinate variation as interchangeable terms to express the idea that "variations" are limited to narrow lines in the individual and in the phylogeny of the race. This use of the term is either for a particular character or for the entire individual. The converse of this concept is expressed by indefinite or indeterminate variation.

These involve two fundamentally different concepts, intended to describe, first, the response to the cause of diversity in the species as a whole or in some isolated portion thereof; second, the limitation of the direction of the response in the individual or in the whole population, in particular qualities, attributes, or conditions. To anyone who is at all acquainted with the conditions of organisms in nature the proposition of Darwin is evident and needs consideration and investigation. Moreover, it is not to be ignored in the consideration and construction of evolution theories, although far too much neglected of late years. On the other hand, the concept that is expressed by definite or determinate as meaning the limitation of departures in the individual or in the race, or as designating trends of evolution in larger or smaller systematic groups, is a no less real and certainly known condition, regardless of what its explanation may be. In these reports I shall use the terms *definite* and *indefinite variation* to designate the method of response as described and used by Darwin, and I propose and shall use in this work the term *delimited* to designate those instances where the modifications resulting are limited to certain restricted paths of change; that is, they are departures whose direction is marked out by physical limitations, impossible

combinations, in the physical material itself. It must be understood that these terms are purely descriptive, indicating only an observed condition in the heterogeneity present in species.

Any particular instance of determinate change may be so because it is also delimited, but not necessarily so; because if determinate, the environment may determine the response, if delimited, the organism. The same kind of organisms might, if undelimited, exhibit determinate variation in different directions in different portions of its natural habitat in a given character, while a delimited character would show determinate variation always the same under different environments. The effect which these relations produce in the population responses of a species is truly important when one considers organisms in nature, and is beautifully demonstrated in some of my experiments in transplanting populations into new environmental complexes. The earlier writings of the Darwinian period much emphasized these variation phenomena, but made no experimental test of their effects.

With the development of modern methods of genetic analysis, attention of necessity was more and more focused upon the individual and upon the characters thereof, so that now our problems are almost entirely those of the individual and its characters and not so much the mass action of the population.

In the development of the mutation theory, De Vries created added complications in the problems of heterogeneity. The familiar assertion that there are really two distinct kinds of variation—mutations (sports) and fluctuations—seems an attempt to separate by definition the two extremes of "variation" which Darwin recognized but was unable to separate. If mutations are the saltation-like changes by which a new unit-character is added or an old one taken away or made latent or recessive, and these steps are multifarious in direction, and if the ordinary "variations" are quantitative, adding to or subtracting from the character in question, then all such variations are always in line, like the swing to and fro of a pendulum, and this is of necessity made so by the definition and the method of description which is mathematical in terms of measure. Associated with this idea is the further assumed difference between the two kinds of variations—that mutations are qualitative and fluctuations are quantitative.

The sharp distinctions made by De Vries have seemed to many a questionable subterfuge by which the unit-character concept becomes more clearly differentiated, and of the two kinds of heterogeneity one, fluctuation, is rendered inoperative in evolution, while mutation bears the entire burden of evolution change and is due to transmutation from present to subsequent unit-characters. Even though this proposition could be shown to be true, it does not follow that fluctuations in a unit-character are always plus and minus, and that the mutations of a unit-character are always multifarious. If there are unit-characters, as conceived by De Vries, each must have relations in space and in interactions, and it is hardly to be supposed that a unit-character is so fixed, so stable and invariable that no matter where placed it is only a little smaller or larger, depending upon the conditions of growth present during its development.

According to Weismann, all variation is ultimately quantitative, due to an increase in the number and vigor of particular determinants, which increase is due to quantitative increase in the determinants. If this be true, it follows that the difference between species, or between their characters, are quantitative,

whereas it has generally been the opinion of biologists that the essential differences between specific living substances are of a qualitative nature. This is important because, if true, quantitative accumulations ought in the end to produce diversity of the kind spoken of as qualitative differences. It raises the question, *can quantitative accumulations in the parts produce resultant qualitative differences of the whole?* This is a problem well adapted to exact experimental test; that is, can the accentuation of an attribute produce a change in the quality of which the attribute is a mass manifestation?

The quantitative concept implies change in the amount of something, with the retention of the same spatial relations; the qualitative implies the establishment of different relations from those hitherto existing. This concept can not be applied at present to the conditioning factors in the germinal material, but it is possible to determine whether the variations are mere plus or minus differences in quantity or material or activities, without reference to spatial relations, or whether the common fluctuations are small differences in the spatial and other relations of the character in question.

The discontinuity which species in nature show is, by this conception, extended to the characters of which organisms are supposed to be composed; whether there is discontinuity between the unit-characters became, therefore, a problem for solution. Because many species show in nature a certain discontinuity in that there are no intergrades, it seems more reasonable to some (Bateson, De Vries) that they arose in that way and that there have never been intergrades, while the fact that there are in nature species groups with intergrades seems to others evidence to maintain that all species groups have arisen through the gradual separation of groups and the extinction of intergrades. In the same way the variations are far too often classed as continuous whenever intergrades can be found and as discontinuous where such are wanting. As a matter of fact, the existence or nonexistence of visible intergrades is of no importance; only intergrading gametic conditions as determined by breeding-tests are of interest. However, in nature the variation conditions in a population are always confused, and mere somatic aberrations, rare chance reconstitutions, and true transmutative variations may easily be confounded and classed as mutations and as discontinuous in character.

Another point upon which there is difference of opinion is raised by the assertion that the only safe basis of study in variation is the "unit-character," meaning thereby some attributes which, as far as is known is not divisible in the organisms, but is present or absent in its entirety. To some these attributes represent the initial unit of structure and activity in the organism, and are, therefore, the only basis from which to proceed in the analysis of organic phenomena, while others hold that the problems must be considered from the point of view of the whole. Naturally these two bodies of workers will arrive at quite different end-results, and not infrequently will fail to appreciate the results obtained by each other. This situation results, naturally, from the difference in the initial philosophical starting-point, and if one's conception of organisms is of the unit-character mosaic variety, unit-characters of necessity become supremely important in the constitution, evolution, and activities of organisms, while if the individual is considered as entity, then all too often that point of view considers only the sum total effect produced on his senses by the particular organism, and his statement of the conditions becomes a sort of impressionistic

one, in which the values and behaviors of the individual qualities and attributes are lost sight of or ignored.

In heterogenetic phenomena, as elsewhere, the unit-character conception seems unnecessary and a cumbersome method of reasoning, and it is essentially vitalistic in its conception. In physical phenomena specific properties are present or absent, are changed with sharp alternativeness, and no need is felt of either such concepts or for inventing mythical pangenes or biophores. If such concepts are not needed in physical science, why introduce them into biology?

For the purpose of this paper I shall consider first those attributes which, as far as known, are not capable of being divided into lesser ones, or simplest characters, and from these shall proceed to the consideration of characters of increasing complexity. It must be understood that this method of attack does not imply in any degree a preference for any current hypothesis. I shall begin with the simplest characters that can be certainly and generally recognized. The criterion for the recognition of this condition has already been stated, and while the criterion will always remain the same, the condition it defines will change from time to time. A name is needed for the conditions from which I start, and throughout this paper I shall call them the "simplest characters," implying no more than the idea already expressed, that they are the simplest conditions that can now be certainly determined and profitably studied among the multitude of specific properties, attributes, and conditions presented by organized bodies.

The development of the "pure-line" hypothesis of Johannsen introduces further complications into the problem. A basal unit or "gene" comparable with "unit-characters," "unit-factor," "element," "allelomorph," and so on, and mainly an expression of the results of the experience of neo-Mendelian hybridology, is conceived of as having a fixed value or manifestation which behaves as a constant factor in organic evolution. The combination of genes in the organic form into a harmoniously acting state of stability, a genotype, is essentially of the same order as De Vries's conception of the constitution of species by combined elementary species. The isolation of genotypes by Johannsen and others, of clones by Jennings, leaves no doubt that there are in the population states of stability which can be separated and maintained as pure homozygous-acting strains. These are always to be distinguished by pedigree breeding methods and are different from "phenotypes," which are separated by purely descriptive methods and may or may not represent "real things."

The genotype conception, while it expresses many experiences in language that is not liable to be misconstrued, nevertheless introduces complications. Opinion, supported by practice in line-breeding, has rapidly established the view that genotypical constitution changes by jumps alone, without intergrades, and that the obvious and constantly present overlapping of genotypes when isolated is only the "fluctuations" of the genodifferences present. The added current belief that no selective accumulation of the fluctuations of the differentiating genes can alter the mean of the "gene," results in much of the work in selection being interpreted as due to the isolation, by "selection," of "genotypes."

With respect to the "gene," as with the "unit-character" or the "simplest character," the essential present need is more real knowledge of these characters. It is necessary to determine whether their commoner differences are mere quantitative oscillations about a mean, whether their rarer jumps to new states are

jumps or not, and whether they are qualitative or quantitative in character. If the gene be so independent and have a mean about which common differences fluctuate, a different conception than is current of those of inorganic bodies must be formed of organic attributes.

Heterogeneity is, of course, the sole basis of evolution, is evolution at work, and the processes which follow are entirely of conservation to preserve, rearrange, and distribute among organic bodies the changed and new attributes presented.

CAUSES OF HETEROGENEITY.

The causes of heterogeneity are certainly diverse, but appear in two main groups of causation: First, transmutation in the qualities with subsequent adjustments in the attributes and conditions of organisms; and second, diversity resulting from recombinations (metathesis).

The opinions concerning the causes of transmutation current with Darwin and his co-workers placed much weight upon the conditions of life as efficient forces for initiating change. This was, however, not much more than opinion and not at all the product of exact investigations. The Weismannian hypothesis also considered the conditions of existence as the essential force in the production of change, but placed the process in a different setting; but the principle was the same—the stress of conditions produced either increase or decrease in the qualities and attributes of the living substance. Some difference of opinion as to how this came about existed, all of hypothetical character and not of any moment here. All saw that in some way there must come to exist in the germ something to give expression to the changed character in subsequent generations. Arguments concerning this method have in the main revolved around the origin peripherally, and the aggregation in the germ, or of the origin directly in the germ and subsequent manifestation in ontogeny. Opinions have differed and still differ with respect to these problems, but in the last decade researches show concretely that transmutations may arise through the direct action of incident forces upon the germ, and that action upon the soma has not produced inheritable changes. These investigations serve in some measure to clarify a much-befogged situation. Clear, consistent proof is provided of the alteration in the germ of that something which conditions a character in the soma, and the evidence available strongly indicates that the changes found are not the product of a struggle between determinants, but changes which appear suddenly, are fixed from the start, and are physico-chemical in character, and not the outcome of a struggle for place and food between lesser organic units.

It is certain that the action of incident conditions is a potent force in transmutation phenomena, but whether it is the only productive force remains to be proved. Many are of the opinion that purely internal operations are in considerable measure responsible for evolution, if not entirely. These automatic internal conditioning and determining forces are dangerous concepts for the biologist. If organisms are conceived of as automatic, internally regulated and propelled mechanisms, they are the only self-contained mechanism in existence, all others depending upon the interplay between the mechanism and external forces for their operation. Even if it be admitted that organisms are automatic, they would, unless possessed of an "inherent tendency to vary," go on in perpetuity in the same form and condition. Inherent tendencies of any kind are

not at present worth consideration, and the attributing of changes to the play between internal parts after all only changes the scenery and not the nature of the play. That is, it is interaction between the something which conditions the character and the materials or forces, or both, external to itself at the point and moment of reaction.

It matters little at present whether I speak of the germ as a whole or of the hypothetical lesser parts; whichever is chosen, the fundamental operation in transmutation is the interaction of the constitution of the gametic substance with the conditions under which its activities go on. Two series of events are ever passing—the antecedent genetic sequence of events in the material composition of the gametes and the sequence of events in its medium; at any moment the constitution of the germinal material and its resultant soma are the product of the dynamic interactions of the two. The two series of events in all instances must be the prime factors in all transmutation phenomena, and they alone are responsible for all transmutative changes of specific properties in organism. If the two series of events are constant, there will be constancy of the resulting organisms; if the organism be plastic in its mechanism and adjusts itself easily to divergent conditions, constancy with considerable adjustive oscillation will result; but if the organic mechanism be little or not at all plastic, divergence in the conditions of its medium will result in adjustments between the mechanism and its medium with change in the mechanism—a transmutative change.

Regardless of what future advances may bring us, the dynamic conception of transmutation provides an easily applied working hypothesis of the cause of change and one that is best expressed at present, though imperfectly, in terms of physical science.

ANALYSIS OF HETEROGENEITY.

One of the most decided advances of recent years is the unquestioned demonstration that any real analysis of the heterogeneity and any expectation of understanding these phenomena must rest upon exact experimental methods of investigation. Biometric analyses, descriptions of phenotypes, have their place as methods of preliminary calibration of materials, but further knowledge depends exclusively upon experimental analysis. Experimental analysis, however, is apt to be pointless unless the results can be directly applied to the understanding and investigation of conditions in nature. Obviously it is neither possible nor profitable to consider all the characters presented, and the results obtained from a few studies will be presented, as illustrating principles common to all specific properties and attributes of organisms.

In the study of heterogeneity of these qualities or specific characters I shall begin with the simplest that I have been able to discover. These, while always parts of a system, are specific in position, form, relations, etc. The differences are often minute and would not ordinarily be used as "specific differences" by taxonomists; however, in all respects they are distinct in character and alternative in behavior in crosses.

CHAPTER VII.

ANALYSIS OF HETEROGENEITY IN SOME SIMPLEST CHARACTERS.

ANALYSIS OF HETEROGENEITY IN SOME SIMPLEST COLOR-CHARACTERS.

It is known beyond doubt that color characters are as fully "law-conforming" in their origin, development, and behavior as structural or physiological features.

In *Leptinotarsa* the color-patterns in the species utilized consist of two superimposed sets of pigments, one in the outer layer of the cuticula, the cuticula pigments, and the other in the tissues of the hypodermis or immediately below it, the hypodermal colors. The relations of these I have described in previous papers. In the formation of color-patterns these two sources of color behave quite independently of each other, the lipoid or hypodermal colors forming the usual color-ground while the cuticular pigments may be either of general distribution over the surface, in which case it may hide completely the lipoid color, or in areas arranged in a precise pattern. The latter colors are always dark browns or blacks, rarely light browns or dark yellow, and may belong, though it is by no means certain, to the melanin series of pigments (Gortner), and arise as the result of the action of an enzyme upon the soft primary cuticula, producing the pigment and the hard outer surface of the integument. The color areas are always developed in precise positions or centers of expression, which, as I have shown, are as fixed in the constitution of the organism as are the relations and positions of nerves or muscles. Many of these areas are simplest characters in that they are indivisible, are present or absent *in toto,* and invariable in position.

I have endeavored to discover what these characters can reveal in the way of concrete information concerning the following problems:

In simplest characters are the differences:

(1) Quantitative or qualitative in character?
(2) Continuous or discontinuous, or both, and what is the relation of the two?
(3) Delimited or undelimited?
(4) Determinate or indeterminate? In restricted localities and groups? In groups and species of wider range?

a, in the adult.—The members of the *lineata* group, save one, all have a spotted pronotum in which the colored areas are developed around a series of centers shown in figure 12. I have elsewhere shown that these centers are invariable in position, but that two or more of them often unite to form a larger color area. Three spots, *c, b, d,* meet all the requirements of simplest characters in that each is always present or absent in its entirety; they are never known to break up into

minor areas or component centers; are common to the species in the group. To varying degrees these areas enter into combination with other areas in the formation of the general color-pattern, especially *b* and *d*, and these combinations I shall consider later.

(1) *Are the differences found in the simplest characters, c, d, and b, quantitative or qualitative?*—The spots meet in a fair way the idea of unit-characters in that they behave quite independently in many processes, and are either present or absent in their entirety; as far as known are indivisible, and in heredity behave as allelomorphs; are present or absent, and the presence of one of these spots in crossing is dominant to its absence, satisfying fully the conception of an allelomorphic pair, but of the simplest kind. The characters meet all of the concepts as to the nature of the most elemental manifestations of gametic constitution as held by the neo-Darwinians. Their invariable position in these animals shows them to be characters whose positions and relations are, and have been for a long time, conditioned in the germinal material. In every way they are comparable to warts, wens, or other extremely localized attributes so often

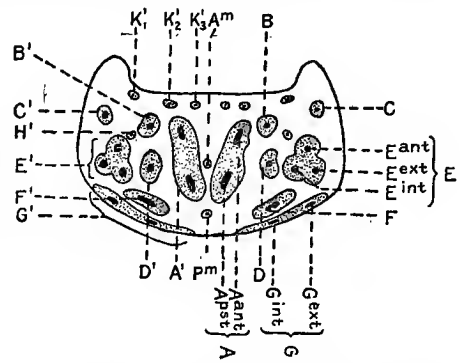


FIG. 12.—Showing the pronotum of the *lineata* group in *Leptinotarsa* and location of the different centers of color formation. The designations are purely empirical, but are those used throughout the text to denote elements of this complex system.

discussed by the Weismannians as certain proof of the existence of conditioning entities in the "germ-plasm," and whose variations, which must form the basis of their transmutations, are "ultimately quantitative."

The question whether the small variations of these characters are quantitative or qualitative is far reaching and of fundamental importance in the analysis of transmutation phenomena, and in heredity and evolution. Putting aside opinions as to the manner in which the various attributes are conditioned in the germinal material, only admitting that they are in some manner so conditioned, the conception of Weismann that all variation is ultimately quantitative is clearly shown in his statement in The Evolution Theory, vol. II, pp. 151-2.

The standpoint that there are two distinct kinds of variation in organisms quite independent of one another, quantitative and qualitative, has been clearly stated by De Vries.

The clearness of De Vries's statements and argument leaves not the slightest room for doubt as to his position and his belief in the existence of two entirely distinct kinds of variation, whose behavior centers around the unit-character. The fluctuations of one of these characters must, by De Vries's proposition, be plus and minus, always in line, the mutations in many directions or multifarious.

The first De Vries considers as due in the main to the conditions of nutrition or an amphimixis-like process, the second to processes at present unknown but having no relation to the first. If the proposition of De Vries is true, then the ordinary or the common intergrading differences of our " simplest characters " ought to be all in line, and this would also be true if Weismann's proposition is true, the chief point of difference being the part these might play in the evolution of organisms.

The first three " simplest characters " for examination, *c, b,* and *d,* are deposits of pigments at invariable positions on this portion of the body; they are *end-products* and as such are the *visible manifestation* of a *specific property* of the *entire mass* in the same way as the angles in the faces of a crystal are specific properties. Spots *c, b,* and *d* have position, dimensions, area, volume, and as a consequence of position sustain exact relations to adjacent parts and characters and present within themselves something of internal organization.

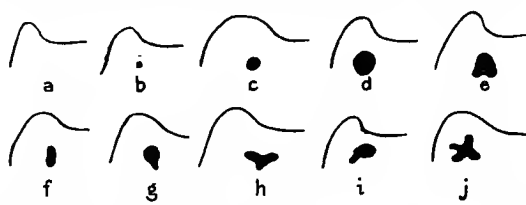


FIG. 13.—Showing variation in shape of spot *c* as it occurs in *L. undecimlineata.* The figures represent examples chosen from many thousands of individuals taken in successive years over an entire known range of species, but show diversity in shape as well as in size that this area may present in this species.

In *L. undecimlineata,* spot *c* is sometimes absent, as in figure 13*a,* but in the great majority of individuals it is present, ranging from the conditions of a minute spot (figure 13*b*) to even larger areas than are shown in figure 13*d.* In any and all individuals the spot is present or absent as a unit, and never breaks up into lesser centers of color deposition. A careful examination of the spots found on the pronota of this species at the center (*c*) shows that they are not all of the same shape, any more than they are of the same size. The difference in size, as far as it is expressed in area exposed, may be a purely quantitative matter, but the shape of the spot is quite another matter. Is this difference in shape quantitative or qualitative?

The simplest condition in which this spot appears is in the form of a small round area of pigment (fig. 13*b,*) and from this as a center the spot pushes out first in one direction, then in another, sometimes in two opposite directions (figures 13*f* and 13*h*), to produce an oblong spot; at other times it pushes forward and outward (fig. 13*i*) and medianward at the same time, or it may push out in four directions, as in figure 13*j.* It is not uncommon for the spot to grow larger without development in any one direction, simply adding pigment equally on all sides, as in figure 13*d.* It must be fully comprehended that these directions in which the spot extends are not in the least purely chance directions, but are firmly fixed in position, delimited in some manner in the physical constitution of the matter involved.

The quantitative aspect of this spot is presented in figure 14, where is given in the form of a curve the distribution of the areas found in the left spot (*c*) in 500 individuals. This statistical data presents simply the fact that in 500 individuals from Tierra Blanca, State of Vera Cruz, Mexico, the area covered ranged from 0 to 3.7 sq. mm., with the modal condition at 1.5 sq. mm. From these data I could, if desired, calculate many mathematical values of no significance. From the *statistical aspect* the variations are in line mere plus and minus additions, made so by the method of study; but examination of the areas shows that there are features that are not and can not be stated statistically; the character has spatial relations, and these can not be analyzed by biometric methods.

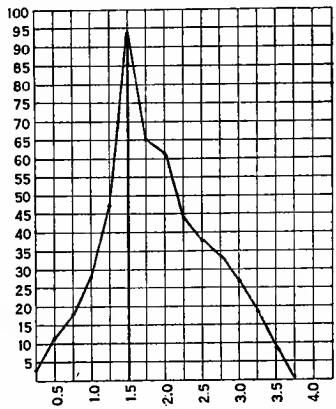


FIG. 14.—Statistical examination of area of 500 left *c* spots in *L. undecimlineata* from Tierra Blanca, Vera Cruz, Mexico.

In figure 15 is given the condition of the spot *c* found in *L. multitæniata* Stål, geographic variety *multitæniata* Stål, at Puebla, Mexico. In this location the spot is nearly always present and usually of considerable area, but shows the same direction of variation as in *L. undecimlineata,* pushing out from the center along the same lines and in the same relative position on the pronotum. Statistically it shows nothing more than in the first case, a distribution of the area of

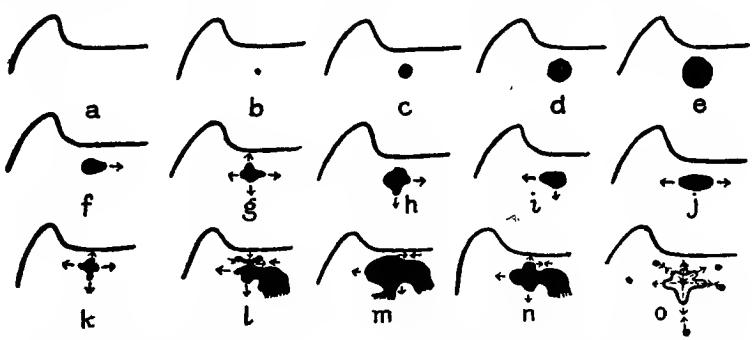


FIG. 15.—Showing difference in size, shape, and directions of extension of spot *c*, in *L. multitæniata* from Puebla.

exposed pigment in a curve extending between different extremes with a different mode, and hence with the possibility of deriving different mathematical coefficients if desired (fig. 16).

The same character in the same species from Guadalupe, Mexico, when examined shows only differences in the end-results, but everywhere the same directions of variation movement. Biometrically considered, the data from 500 individuals show a different mode and distribution of areas (fig. 17). One might go on in this manner without limit and in all it would be found that when the areas were measured the variations would *all fall into line, plus and minus.*

It is only in one very trivial aspect, however, that the differences in spot *c* conform to this condition, as has been shown in the three sets of examples given. Area in this character is not necessarily quantity of pigment, but area of surface pigmented, and can not show truthfully either the quantity, process, product, or distribution in either materials, duration of processes of pigment development, or relations to adjacent parts.

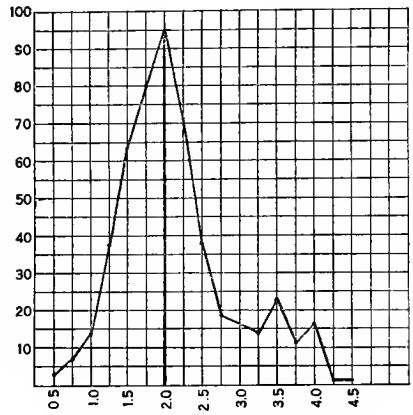


FIG. 16.—Area of 500 left *c* spots in *L. multitæniata* from Puebla.

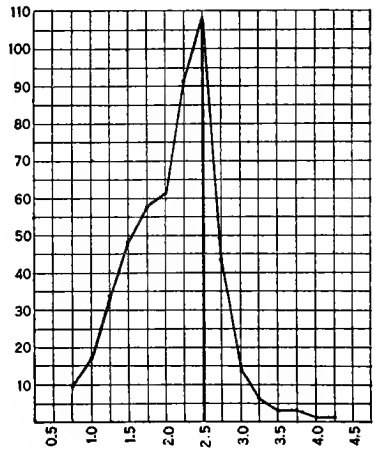


FIG. 17.—Area of 500 left *c* spots in *L. multitæniata* from Guadalupe.

If the distribution of the pigment in the spot *c* is considered, and not the amount of pigment, it appears that there are several directions of change in the distribution of pigment and in relation of these to the other centers of coloration. Is it the amount of the pigment, the area colored, that is important, or is it the arrangement and changes in arrangement and relation to adjacent parts that are important? Spot *c* presents two distinct sets of "variation" data, of

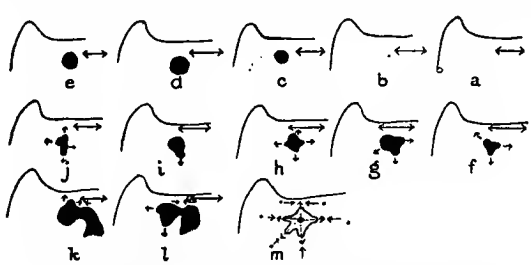


FIG. 18.—Showing difference in size, shape, and direction of variation in spot *b* in *L. undecimlineata*.

which the relative importance is entirely dependent upon one's initial philosophical viewpoint, upon definition. Other similar areas of pigmentation on the animal show identical conditions in principle and confirm in every way the statement made. Different manifestations of spot *b* on the pronotum of *L. undecimlineata* are represented in figure 18, and show that the

spot may be entirely absent or present as a simple round area and that it may retain its round form and increase greatly in size. As in the spot *c* it also changes now in one direction, now in another, or in two or more at the same time, but these directions are never chance or haphazard, but always are along certain invariable lines. Statistically, a lot of 500 *b* spots shows a typical curve of distribution, plus and minus (fig. 19). In *L. multitæniata* spot *b* shows the same condition present or absent in its entirety, present in the simplest condition as a simple round spot, often of considerable size as a round spot; at other times pushing out in different directions, but never in chance or irregular directions. The statistical statement from a lot of 500 left spots offers nothing of interest; it is simply the plotting of a lot of areas having upper and lower limits—a mode, a mean, and coefficients not worth calculating.

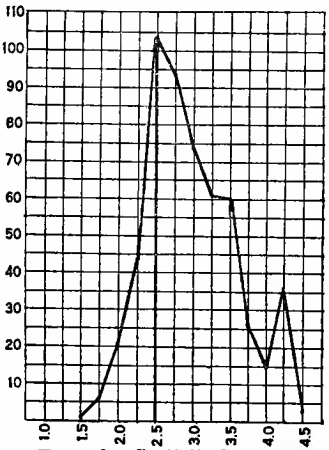


FIG. 19.—Statistical treatment of a uniform lot of 500 spot *b* areas in *L. undecimlineata* from Tierra Blanca.

It would be easy to go on endlessly accumulating data of this kind, and I could fill many pages with the mere statement of the facts gathered concerning spots occupying the position 16*c* in chrysomelidæ, and also in other families of Coleoptera. Naturally, not all show the same conditions, but all give the same results in principle, namely, that the common variations of these simple indivisible color-characters present two superficially distinct variation phenomena—diversity with respect to area in units of some measure, and in the arrangement and relation of the character, both external and internal.

It is obvious from the data given that the common differences of simplest characters are not all in line, plus and minus, but that there are changes first in one direction, then in another, or in one or more directions at the same time. If the biometric definition of fluctuations be true, then all changes found that are not plus and minus in terms of quantity, but are multifarious in direction and rela-

FIG. 20.—Spots having the same area, but not the same shape or direction of variation; statistically unlike things are often confused on the basis of measurement and treated as like.

tion, are mutations, and only those measurements of the area of pigmented surface are fluctuations and are of necessity in line, because forced there by the method of description. Area, moreover, is not an accurate method of describing the condition of these spots, and this I have shown in figure 20, where there are shown spots all of the same measured area, but their shape is quite diverse and might be productive of quite diverse results. Statistical methods force these spots into the same class and count them in statistical analysis as all alike. *They are decidedly unlike.*

If the changes in different directions producing unlike shapes of spots are mutations, there ought to be visible or demonstrable discontinuity, but there is nowhere the slightest trace of breaks to be discovered. The "variations" which these spots show are not of the same kind, or at least have different directions and hence differing consequences in the composition of the color-pattern of the part. Movement medianward is different and is productive of different pattern-results from one that moves caudalward. The departures shown by these spots in several directions can be found in many if not in all simplest characters, showing first one, then another, or one or more of its potential possibilities at the same time. I see no escape from the conclusion that the biometric definition of fluctuations is not only not true as a general proposition concerning variation in simplest characters, but must be regarded as an anticipatory definition framed in the interest of a particular hypothesis, and in no wise a carefully derived conclusion directly deduced from data carefully considered.

It is possible that supporters of the biometric proposition may maintain that the different directions of variation found are in reality the basal "unit-characters," and that variations along the different lines are the variations to be considered, and statistically analyzed. Experience has not shown these spots ever to break up into lesser units in nature, and I have not been able to accomplish it by experimental means. Further than this I can not go at present; as it represents the limit of experience and the conclusions to be logically derived therefrom. Any further statement would, therefore, be anticipatory, dogmatic, to support some one or other theory, but any other similar statement has just the same chances of being true or false. It must be shown that the simplest characters in nature and in experiment can be broken up into lesser units, and until this is done it is fairest to consider that spots *c*, *b*, and *d* really represent simplest characters, indivisible, and that they clearly do not conform to the requirements of the De Vriesian hypothesis as regards their common or fluctuating variations.

The question whether the pigment as a substance which should be measured in terms of quantity or area colored and the pattern or distribution of it considered as another character, and that the two are necessary for visibility can hardly be answered with any exactness. Neo-Mendelian hybridology has shown clearly that pigment and pattern can be shifted independently of one another in crossing. If this proposition be admitted as true and as applicable to these simplest characters, the problem is not solved. Pigment then becomes a character that fluctuates in quantity as statistically measured; pattern, its companion, shows nothing that can be called fluctuation, nor that can be called mutation. The decision would largely depend upon definition in the absence of experimental evidence.

Each of these characters presents, therefore, heterogeneity; and depending upon what is considered the correct point of view hangs the kind of statements that will be made. If the character is calibrated only in units of area, diameters, length of perimeter, and angles between the directions of variation, the statements will all of necessity be in terms of more or less; the determinations are forced to become quantitative. *What is actually present are differences existing between the same character in different individuals, and these are neither entirely quantitative nor entirely qualitative, but are differences in the quantity of material, in composition, and in the physical position, arrangement, and relations to surrounding parts.* From a descriptive standpoint it is impossible to go further.

It would be rash, with present knowledge, to attempt further separation which would be purely anticipatory as to the nature and constitution of organisms. I believe the conclusion valid that there is no sharp demarcation in nature. Aspects of any character that are measured in units of some measure will be arrayed in line and expressed in terms of measure; those that are not or can not be measured must be expressed in terms of position and relationship; and there the matter rests for the present. It is not possible to express in quantitative terms, plus and minus, the facts of the fluctuations of these single characters.

(2) *Are the differences of the simplest characters, spots c, b, and d, continuous or discontinuous, or both, and what is the relation between the two?* In recent years many authors have drawn sharp distinction between continuous and discontinuous " variations " and their supposed rôle in evolution. That variations do arise suddenly is undeniable, at a single step, and stand much apart from their fellows without intergrading conditions; and from changes of this kind stable races unquestionably have arisen. As a protest against the complacent assertions regarding the omnipotent efficiency of minute, constantly accumulating, utilitarian variations as the sole cause of evolution, and in the hope that a new viewpoint and mode of investigation might be the outcome, many have enthusiastically advocated the idea that sudden transformations in the attributes of animals and plants accounts better for the rise of stable races and species than slow accumulation of utilitarian variations. Discontinuities, or sudden jumps with gaps wherein there are no intergrades, must of necessity follow in De Vries's system, from the very nature of the initial concept as to the nature of organic bodies. Regardless of the assumed underlying entities, actual investigations of the origin and behavior of these sudden variations have been organized and completed with marked success, in striking contrast to the masterly inactivity of the neo-Darwinians. These variations in most instances are the same as the " sports " or " spontaneous variations " of Darwin and Wallace, the " saltations " of other writers; but in De Vries's oenotheras a different phenomenon is clearly present.

It is necessary to keep constantly in mind the possible differences in these changes; one arising as " variations " in the sense of a departure; the other as due to rearrangements of characters following crossings. De Vries's oenotheras, in their recurrent production of the same types, clearly suggest this origin for their " mutations," and the recent studies of Davis, Herbert-Nilsson, Gates on *Oenothera,* and mine on *Leptinotarsa,* show clearly the fact and a method of producing these mutating races.

It is shown that the fluctuations of simple characters were not linear, but in several directions, and that there were also variations in amount, extent of area, and other measurable features; in other words, that there was a complex variation of the simplest character.

In the study of the variations of the simplest characters many conditions have been found which might be described as discontinuous, where in the population there were no intergrades. One of the most common discontinuities is that found in statistical study, of which one illustration is presented in figure 21, where in 1000 left *c* spots of *L. undecimlineata* Stål, from Tierra Blanca, Mexico, there were a few individuals that stood some distance from the rest of the variates of the lot and without intergrades. These latter were all collected

from one small plant, in a very moist situation, and were noted at the time of collection, and on measuring the lot these individuals stood out from the rest as a group by themselves. Two months later, in the same location, I collected a second lot of 1000 (the next generation) and examined the left *c* spots statistically. The results of this examination are presented in figure 21, where the isolated group found in the first curve of distribution is also present, but in this case it is included in the curve of continuous variations. Another set of observations made at a point near San Marcos, in Vera Cruz, Mexico, gave different data, but results that were identical in principle in that the data of the variability of

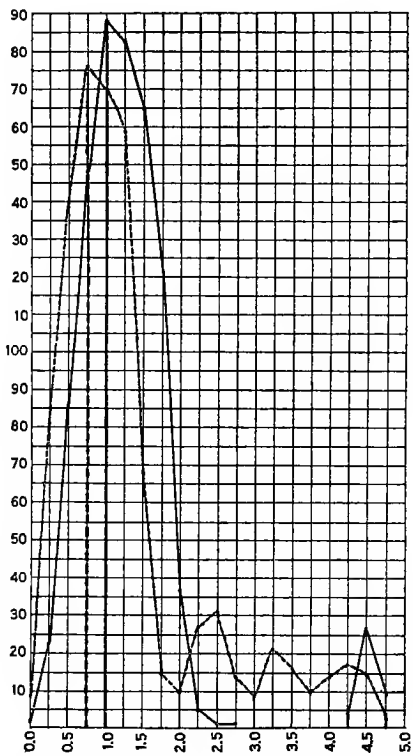


FIG. 21.—Measurements of left spot *c* in *L. undecimlineata* at Tierra Blanca to show differences in the array in two successive generations.

the left *c* spot in 500 individuals gave a polygon of distribution with a small group on one side, but connected with it by intergrades. A collection in exactly the same locality in the next generation gave a different condition in the polygon of distribution, with the group standing below the general population entirely cut off, and intergrades were wanting. This extreme group came entirely from an area of coarse gravel with little loam, and dry at all times. The first collection was made in the early part of the rainy season, when it had been rather dry over the plain as a whole, so that there was a rather continuous series of stages of dryness from the extreme state of the gravel bed to that of the rich soil of the

savannah; and the second lot was collected later in the season at a time when it was raining daily, the only dry place was the gravel-bed, and here the individuals were found that were discontinuous from the general population. These statistical discontinuities can be multiplied without number, and are sharp changes, often only produced in a few individuals by chance conditions of location, but the majority of the population was not so influenced. It was not observed that the influence had produced any change in the shape of the spots or in the different directions in which this spot varied; it was simply in one case an accentuation and in the other a diminution in the amount of the pigments formed as measured by the area darkened by them. Both were simple climatic aberrations and on testing failed to appear in the next generation so that statistical discontinuity or aberration means little or nothing.

Is there any discontinuity in the presence and absence of these spots? Careful examination shows the gradual diminution of any or all of these spots until the traces are no longer visible to the unaided eye, but proper preparation shows on microscopic examination a long scale of decreasing color values. In their lowest terms these simplest characters may be invisible to the eye, only microscopic traces persisting, often a faint light yellow-brown color in the lower portion of the cuticula which gradually vanishes completely. In these characters there is no visible discontinuity in the present and absent conditions; nevertheless the two extreme conditions are capable of fixation in "lines," and when crossed these show clear dominance of the presence over the absence and segregation in F_2.

There is divergence between the different directions of variation of these spots. Spot *b* varies in an antero-posterior and in a medianward direction, rarely in others. Between these directions of variation no intermediate directions have been found. Occasionally a direction not hitherto observed arises suddenly in some individual or individuals. The new direction of variation starts from the center and goes in its own direction with remarkable precision. There is divergence in the directions of change in these characters but whether there is discontinuity depends upon definition.

In this section the answer to the question turns in a way not unlike that of the first section; there is diversity, divergence, with discontinuity of end-results of diverse sorts, but the proposition of the unity of direction in "continuous variations" seems entirely unfounded. There are statistical discontinuities, but from the method of examination these can exist only in plus and minus aberrations; and further, this method of statement "lumps" so many different conditions as one homogeneous class that it is extremely doubtful if the results of statistical study are in reality biological at all. There remains the undoubted fact that in these simplest characters there are differences that are continuous, in that there are plenty of intergrades, and those that are divergent, giving discontinuous end-results between which present knowledge has found no intermediates. Actually, the "variations" are diverse relations in constitution, amount, space relations, and interactions with the rest of the mechanism, which result in differing arrangements in the visible product deposited as the outcome of these processes. That intergrades are present in some aspects of the complex, lacking in others, is not a basis for the creation of sharply marked categories. That intergrades are present or absent is no criterion of the nature of the processes, and it

has not yet been shown that in any of these simplest characters, or any of the so-called unit-characters, there is a different composition where there is statistical discontinuity.

(3) *Are the variations of the simplest characters delimited or undelimited in the spots c, b, and d?* The answer to this question has been to a considerable extent given in the preceding sections. All of the variations thus far seen are delimited in that they are all confined to certain well-marked directions of variation. These spots do not vary at random, but in conformity to certain rules; what these are need not now be stated, as it does not affect the situation.

The delimited nature of the variations of these simplest characters shows certainly that they are not homogeneous or undifferentiated things, but each has structure and relations that are its own, and entirely characteristic of it. That is, in the areas called spot *c* or *b* or *d* there are forces and physical relations existing which center in the location *c, b, d,* and the same is true of all other color areas in these organisms, which control the distribution of the pigment and limit it to certain positions, which means that the underlying processes producing the pigment are themselves thus localized and vary. For convenience of expression it can be said that each of these spots has an organization peculiar to itself, which delimits the variations which it shows, but it must be clearly understood that this does not mean that the spot exists as an entity, independent of the rest of the pronotum. Experience gives no indication of any such condition. All that is known is the fact of the localization of the results of certain processes at a fixed point on the surface of the pronotum, which there carry out certain reactions from which, as a product, pigment is produced and distributed in the same position as the processes themselves are localized. These localizing conditions, as far as it is possible to analyze them, seem to be properties of the whole organism presenting most exact and complex arrangements.

It is important to recognize that in these minute and unimportant simple characters the variations, to use Eimer's phrase, are " law conforming "; that is, are orderly and not disorderly. This signifies of course a complete control of the conditions in the smallest of the attributes that it is profitable at present to study. These characters in organisms stand in the same relation to the organic body as do angles, hardness, color, etc., in inorganic bodies. They are not entities in a mosaic, but manifestations of physical and chemical interactions giving as an end-result the things seen and used as a biological alphabet.

(4) *In local areas and in species as a whole, are the variations of the simplest characters definite or indefinite?* Whether variations are definite or indefinite in Darwin's meaning of the term is a question of prime importance in the solution of many problems in organic evolution, especially in the formation of groups and species in nature, and has been altogether neglected in recent years.

In Darwin's conception of this response in " variation " there is a broader recognition of the general relationships of " variation " phenomena in organisms that is found in the writings of his critics, or in any of his professed followers. This aspect of the study of variation has been tested in spots *c, b,* and *d* in *L. undecimlineata* Stål, *L. multitæniata* Stål, *L. oblongata* n. sp., *L. decemlineata* Say, *L. signaticollis* Stål, and *L. diversa* n. sp., and in all the results have been the same in principle, different only in statistical details. I shall present the data of a few of the studies for consideration.

Statistically, the problem must be answered from four aspects: (1) in the species as a whole, regardless of where or when the specimens lived, it is necessary to determine the entire range presented; (2) comparisons of one locality with another are necessary to discover possible local influence; (3) successive generations in the same location must be compared to determine the constancy of relation between local causes and effects; (4) comparison of series of generations in the same and different localities to determine the existence of continued or permanent local influences. These four examinations will give all the information possible from this source, and when combined, will give some basis for the determination of the nature of diversity in these simplest characters, and provide the proper data for initiating experimental analysis of problems not touched by the methods used. In this study I have examined these characters in many thousands of specimens, and in living material and museum specimens have looked constantly for new or unusual " variations." In figure 22 I have shown outline drawings of the main types of variations found in *L. undecimlineata.*

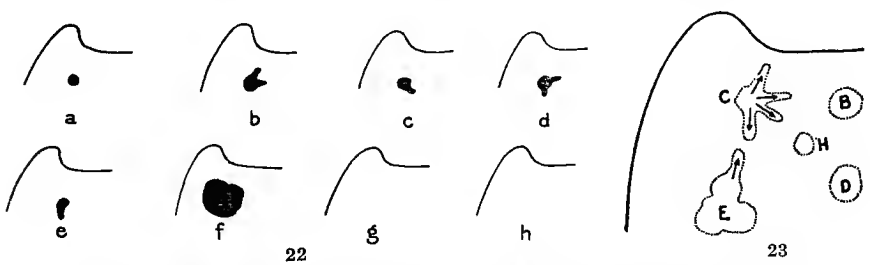


FIG. 22.—Diagrammatic representation showing common types of variation in spot *c* found in *L. undecimlineata.*

FIG. 23.—Diagrammatic representation of outer portion of pronotum in *L. undecimlineata,* showing relation of different directions of variation in spots *c, e,* and relations of these to the element *x.*

Examination of the variations shows clearly that the character in its variations is delimited to a few directions of change. These are diagrammatically shown in figure 23, which is a composite of the spot in outline to show the direction of departure. As compared with other species, it is quite distinct in its variations, which are few and rarely of any magnitude. Medianward fusions with *b* have not been found, although *c* may extend towards *b,* but *b* does not move towards *c;* hence there is no fusion. Caudalward unions with *e* are rare, and result from combined action of *c* and *e ant.* towards each other; and still more rare are fusions with *d,* which depend upon the presence of a rare element *h.* Its absence is not infrequent and its presence never reaches an area of over 5 sq. mm., 4.89 sq. mm. being the largest seen, and that a product of selection plus environmental stimulus.

Parallel observations in nature were made near Tierra Blanca, 57 miles south of the City of Vera Cruz, at El Hule, 39 miles south of Tierra Blanca, and at San Marcos, 68 miles south of El Hule, all upon the savannahs of Vera Cruz, Mexico.

The location at Tierra Blanca was on the edge of a small stream 3 miles from the town on the trail of Estanzeula. The immediate location was typical tropical savannah with thorn forest, well watered, good drainage, and about 225 feet above sea-level. The El Hule location was on the southern edge of the flood-plain of the Rio Papalaopan, west of the station buildings about 1 mile, on the

trail of Tuxtepec; also typical savannah but more open and park-like, fewer trees and many annual and shrubby perennials, and about 300 feet above sea-level. At San Marcos, 600 feet above sea-level, the location was about 1.5 miles west of the station on the eastern edge of a rainy season lake. The area was savannah, but with tree islands, poorly developed drainage, abundantly watered and covered with short grass and low herbaceous plants.

At all three places the annual cycle of seasons is the same in type but differs in details. Recording thermometers that were secreted in the open and read on returning gave the highest and lowest temperature that had elapsed since the last observation. Tierra Blanca gave the highest temperature, 107° F. in the shade, May 1906, and the lowest record obtained there was 54° F. in December 1905, during a "norther." San Marcos was always cooler, the extreme records being 97°F. in May 1906, and 45°F. in February 1906. El Hule was more moist and always had fairly high humidities at all seasons, while Tierra Blanca had the least rain and the lowest humidities.

The plan adopted was to go to each location at proper seasons and there make determinations of the value of at least 500 individuals. Owing to the peculiar localized character of these colonies, none, or at least only a few, were removed because it early developed that artificial removal soon altered the tone of the local group under observation. To have removed 500 would often have taken nearly the entire population of the place. Usually one or two natives were employed to gather the specimens, while I determined and recorded the values. To facilitate this, scales of areas were made as a basis of comparison, and the material seriated quantitatively, and also camera outlines were made and measured by planimeter later. Either method gave the same results, the latter more detailed, but no more accurate or significant. Variations in shape were recorded in terms of type of variation. Two sets of observations were obtained, therefore, at each location, and for each period of observation a quantitative measure and an expression of the frequency of different types of distribution of the pigment and the variation directions of the spot. As a rule, two to four days at each place were necessary to obtain the requisite number of records. No specimens were returned to nature until after the records were completed.

The results obtained in the years 1905, 1906, 1907, 1908, and 1909 are presented in the condensed form of tables.

Quantitatively, the San Marcos group showed (table 23) constantly in all of the ten determinations the least amount of pigment, but a considerable variation range, while the Tierra Blanca group gave constantly the largest amounts of pigment, and also considerable range of variation; the El Hule determinations gave the greatest variability and the least constancy in values. In all it is clear that the members of the population responded as a mass to the conditions of life, in that the polygon moves as a whole plus and minus in different determinations. From quantitative determination the conclusion might be drawn that quantity varied determinately in this character, but it does not give any basis for a discussion of its action in evolution until it is shown whether the variation is permanent or can be made so. Statistics can not determine this, but they might give the basis upon which to base experimental operations. The variation differences found in these locations may be due to different external conditions, to localized racial characters resulting from partial isolation, and

TABLE 23.

When observed.	Values and limits of classes in area (sq. mm.)																					No.
	0	.01–.25	.26–.50	.51–.75	.75–1.00	1.01–1.25	1.26–1.50	1.51–1.75	1.76–2.00	2.01–2.25	2.26–2.50	2.51–2.75	2.76–3.00	3.01–3.25	3.26–3.50	3.51–3.75	3.75–4.00	4.01–4.25	4.26–4.50	4.51–4.75	4.76–5.00	5.01–5.25	
Tierra Blanca.																						
1905. June	6	27	41	86	131	126	77	50	24	10	6	18	7	2	9	1	...	...	...	...	...	621
August	...	...	1	7	5	43	98	151	114	91	36	10	2	...	...	...	...	...	...	...	...	558
1906. June	...	4	14	41	96	102	119	174	131	77	55	24	10	3	...	...	...	...	...	...	...	850
August	5	9	31	77	141	219	186	91	49	19	9	7	7	4	2	2	2	1	...	...	...	861
1907. June	...	...	3	18	46	118	201	141	77	12	4	...	...	...	...	...	...	...	...	...	...	620
August	...	2	1	9	37	45	87	196	95	18	10	7	17	4	9	8	5	9	2	1	...	562
1908. June	...	...	...	7	46	105	119	100	41	38	19	10	2	...	...	...	...	...	...	...	...	487
August	...	...	...	5	18	96	304	201	77	18	6	4	...	...	9	18	31	20	4	...	...	811
1909. June	...	1	6	7	17	44	98	109	91	51	37	26	21	19	8	9	3	2	...	...	...	549
September	4	...	...	9	31	96	208	109	77	61	43	19	12	4	3	1	...	...	...	...	...	677
El Hule.																						
1905. June	10	4	17	31	44	75	69	84	31	41	11	14	5	8	4	7	2	...	...	...	...	457
August	...	7	9	46	17	83	77	72	67	59	34	21	17	10	5	11	4	6	2	...	...	547
1906. June	32	46	86	105	24	35	46	12	31	36	29	19	11	4	6	3	8	2	4	7	...	550
August	7	...	...	5	9	41	96	77	74	39	31	20	10	4	...	...	...	...	...	...	...	413
1907. June	19	21	34	46	92	90	81	70	50	44	20	14	11	6	...	7	3	...	...	...	...	608
August	4	6	5	8	14	46	89	147	134	125	81	44	30	10	4	3	1	1	2	1	...	755
1908. June	2	2	4	9	31	46	84	49	31	10	4	3	1	1	4	2	1	...	...	...	...	284
August	1	1	2	96	106	41	21	10	4	1	...	...	...	...	...	...	...	...	...	...	...	283
1909. June	...	...	4	7	38	99	301	204	106	61	50	44	18	10	2	...	...	...	...	...	...	944
September	17	12	86	210	146	191	130	98	75	55	40	21	16	13	11	8	10	4	7	1	...	1,151
San Marcos.																						
1905. June	10	47	96	219	84	51	20	9	4	4	5	6	4	2	1	1	1	1	1	...	...	566
August	6	31	144	77	31	19	38	12	19	4	3	1	...	1	...	2	...	1	...	...	...	389
1906. June	1	77	210	191	77	44	6	4	2	2	...	...	4	5	...	1	2	...	...	...	...	626
August	31	196	131	79	61	33	25	9	7	18	4	6	1	...	...	...	...	...	...	...	...	601
1907. June	4	21	84	97	101	310	99	44	19	6	...	1	3	...	7	1	...	...	...	...	...	797
August	7	47	96	209	100	44	10	39	41	7	9	...	...	...	...	...	...	...	...	...	...	609
1908. June	31	86	210	144	96	45	19	18	31	10	14	2	3	5	9	11	3	5	6	1	4	753
August	1	44	96	31	22	18	6	9	...	...	...	1	...	1	...	2	...	...	...	...	...	231
1909. June	21	35	76	119	24	46	92	17	21	10	5	...	...	...	...	...	...	...	...	...	...	466
September	7	18	44	75	114	195	151	91	76	45	17	22	12	11	8	7	4	...	1	...	...	897
																					Total examined	17,723

so on. They can be either somatic or germinal, but no trace of knowledge of this either one way or the other is found from the biometric analysis. From any such body of data any one of many " conclusions " can be drawn, but in all the conclusion is an assumption made to interpret the array of mathematical values which the biometric method has obtained. The method shows a condition only,

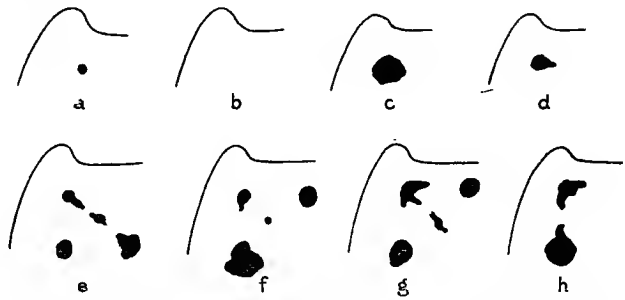


FIG. 24.—Showing directions of variation found in spots c, *x*, *d*, and *e*, in *L. panamensis*.

and none too accurately, because it can not distinguish germinal and ontogenetic differences, or any other kind of heterogeneity present. It is, however, a fairly good crude method in obtaining preliminary data of conditions in a population.

Examination of the directions of diversity found in *L. panamensis* (fig. 24) shows differences in the areas, but the general result is clearly a delimited variation. In restricted localities the population shows remarkable delimited movement of the population as a whole in certain directions. To what agencies these movements and responses are due is not determined by the methods of the census; an endless array of possible causes for the observed effects could be suggested and plausibilities put together to no purpose whatever.

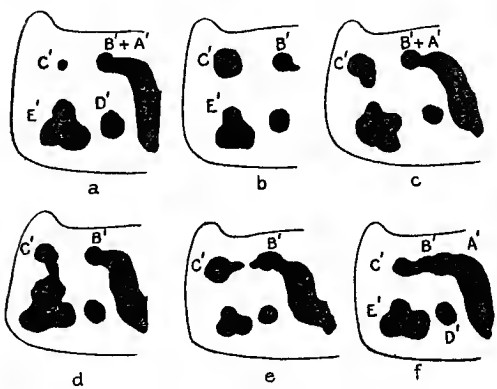


FIG. 25.—Showing directions of variation, especially in spots c, *b*, and *e*, in *L. signaticollis*, and some results which these directions of variation may produce in the production of pattern differences.

In *L. signaticollis* the same spot (*c*) occurs and shows some of the same directions of variation as in *L. undecimlineata*, but in area it much exceeds the first species. In figure 25 are shown the variations thus far discovered, together with the results produced in the pattern. In this species spot *c* is most commonly present as a round area of variable size, is never absent, with the observed

extremes as represented in figure 38, *a* and *b*. It may extend medianward towards *b*, but this is always accompanied by a lateralward movement from *b* towards *c*. These two always move toward each other at the same time, producing a mutual movement to unite. Likewise *c* and *e ant.* may simultaneously move towards each other and unite. The movements of *c* and *b* towards each other, of *e* and *f*, and the movements of *c* and *e ant.* are shown in figure 25. These states are inheritable in pure homozygous biotypic lines and are alternative to their absence in crosses. No other variations in direction are known in spot *c* in *L. signaticollis*, either in nature or in cultures, and none has thus far been induced by experimental means.

The biometric study of this area shows little of interest. In figure 26 are given the statistical determinations for the populations in restricted colonies at

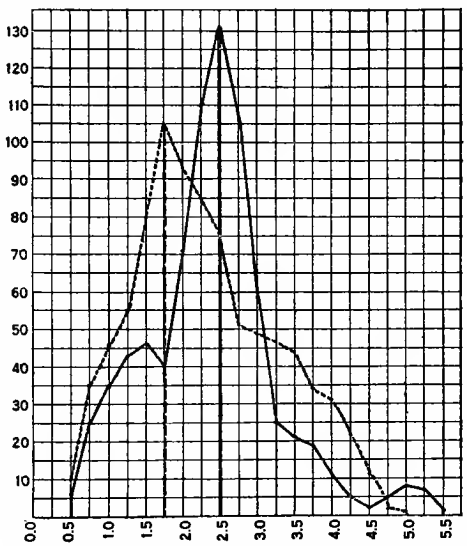


FIG. 26.—Biometric treatment of the left spot *c* in *L. signaticollis* at Cuernavaca and at Atlixco.

Atlixco, in the State of Puebla, and Cuernavaca, in the State of Morelos, Mexico. In the Cuernavaca colony determinations of left *c* spots were made in 1904, 1905, and 1906, and show a mode and a wide range, but the spot is never absent. In the Atlixco population for the same time is shown a lower mode. Both modes are higher than the modes for this character in *L. undecimlineata*, and the mode for Cuernavaca is the highest known and occurs only in one restricted area. Other colonies in the Cuernavaca Valley have much lower modes.

Biometric observations upon the state of the population at these colonies in the Cuernavaca area were made in 1904 to 1908, a total of ten observed generations. The three colonies were different in conditions, especially in the water-relation. The Bosoco colony was located in the shallow, steep-sided barranca (50 to 100 feet deep) to the east of the town, and so located that the deep, rich soil was moist, due to drainage conditions. The Quauhtemotzin colony was on

TABLE 24.

	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
Quauhtemotzin Colony	3	3	5	12	21	77	98	141	67	17	3	2	
Basoco Colony	2	4	11	31	89	**165**	92	26	5	1	2	1	
San Antonio Colony	1	6	178	**211**	183	92	35	6	1				

TABLE 25.—*Observed frequency of occurrence of the different types of variation in spot c in each of the colonies during ten successive "generations."*

	Observed shape and direction of spot *c*.						
	Absent.	Small.	Large.	*c*→*d*.	*c* + *d*.	*c*→*e*.	*c* + *e*.
Basoco Colony:							
June, 1904	...	17	171	39	31	1	...
August, 1904	...	24	186	51	15	5	3
June, 1905	...	51	77	5	3	...	...
August, 1905	...	106	118	29	2	...	...
July, 1906	...	77	71	14	5	4	...
September, 1906	...	15	37	10	2	1	1
July, 1907	...	49	95	34	15	6	4
August, 1907	...	74	83	41	...	4	...
June, 1908	...	10	21	15	...	2	...
August, 1908	...	92	109	4	...	...	...
Quauhtemotzin Colony:							
June, 1904	...	142	214	12	1	4	1
August, 1904	...	171	186	19	...	3	...
June, 1905	...	86	92	4	...	...	...
August, 1905	...	105	77	10	...	1	...
July, 1906	...	88	31	25	2	...	1
September, 1906	...	21	36	15	3	1	...
July, 1907	...	55	67	10	...	4	4
August, 1907	...	71	175	36	2	19	7
June, 1908	...	45	56	12	...	5	3
August, 1908	...	109	211	71	...	6	14
San Antonio Colony:							
June, 1904	...	71	281	...	...	14	2
August, 1904	...	121	145	...	...	19	17
June, 1905	...	59	314	...	...	71	4
August, 1905	...	50	49	...	...	3	7
July, 1906	...	92	86	...	...	14	9
September, 1906	...	46	92	1	...	19	17
July, 1907	...	31	55	...	...	4	...
August, 1907	...	100	210	...	...	6	2
June, 1908	...	18	37	...	...	4	3
August, 1908	...	77	88	1	...	10	2

the eastern slope of a large barranca and always moist, owing to constant seepage of ground-water. The San Antonio colony was located on the west side of the river above the Falls of San Antonio, and was the best drained and driest of the three. All were about the same altitude and received the same rainfall. Evaporation, however, was most intense at the San Antonio, least at the Quauhtemotzin colony. Statistical determinations made by measurement and seriation were used, frequently together, and the results show constant differences between the three locations, between which there was little chance of interchange. They have not been found to cross the elevated ridges from colony to colony, but work up and down the barrancas, so there was little or no intercommunication probable and none was discovered between them. In table 24 are given the results from these determinations. It is evident that the Quauhtemotzin colony has always the highest mode, that is, *c* is larger here than in either the Basoco colony or the San Antonio colony, where it is smallest. As far as the biometrics are concerned, here the story ends. No more information of any importance is forthcoming as the result of the application of this method of study. The conditions and any mass actions of spot *c* in the population are known; the reasons for these conditions can only be analyzed through experiment. At best, only a *plausible interpretation* of the observed condition based upon a favored assumption is possible, which is neither proven nor investigated; and to assume that large water-content in the medium means much pigmentation and lesser amounts mean less pigment is nonsense. This relation of water-content may be an entirely correct conclusion, but by no means do the biometric data in this or similar instances prove the conclusion at all.

TABLE 26.—*Percentages of fusion or movements to produce fusion in the total population of the Cuernavaca district that were observed in the three colonies at Rancho Basoco, San Antonio, and Quauhtemotzin colonies with respect to directions of fusion.*

Source of the stock.	Round, either small or large.	c⇄b or c+b.	c⇄e or c+e.
	Per cent.	*Per cent.*	*Per cent.*
Basoco colony	23	5—	0.5
Quauhtemotzin colony	32—	2.9—	1.—
San Antonio colony	31+	.....	3.—

Much more interesting and indicative of more significant conditions in the population are the results from a census of the directions of variation of spot *c* in the three locations. This I have shown in table 25, where the census is recorded for each of the three stations at Cuernavaca for 10 consecutive periods of observation. In this census a decided difference is evident in the San Antonio colony, in that $c \times b$ is not found, and its presence is only indicated in 2 females, neither of which was able to transmit the observed variation. Both were tested by breeding. At the Basoco and Quauhtemotzin colonies all the variations were found, but in different populations. In all, 6377 records were made at the three colonies in the 10 censuses. Regarding this as the population of the Cuernavaca area, the three local populations show their differences in percentages of the whole as shown in table 26.

Table 26 shows local diversity in the directions of variation, and they may be, as far as biometrics go, either germinal or somatic. Experimental testing, aided by adequate field observations alone, can decide, and this I have done in this instance by bringing stocks from all of the three local populations to Chicago, where under favorable conditions of culture they thrived and gave a combined result shown in the table 27.

TABLE 27.—*Results found in stocks from three Cuernavaca District colonies, when reared under like conditions at Chicago.*

Source of the stock.	Small or large.	c↔d or c+d.	c↔e or c+e.	No. of generations.
	Per cent.	*Per cent.*	*Per cent.*	
Basoco colony	25 +	7 +	1 +	8
Quauhtemotzin colony	29 +	2 +	2 +	6
San Antonio colony.....	30 +	....	3.5 +	5

It is certain from this test that the three stocks are different in gametic constitution; otherwise why should they retain, under like conditions, when grown side by side, the same differences observed in nature and tested in this simple experiment? The result shows the fixity and specificity of these minute and trivial characters in the organisms, and also the *delimited, determinated response* in the *directions* of heterogeneity in the different locations, but to what agency this determinate response is to be attributed is not indicated. In the amount of pigmented area perhaps more certain determinate response is observed, but this is of no significance.

In *L. diversa* and its geographic variety *rugosa,* little need be said. No directions of variation are found, and it has not been observed to be absent in any individual that was able to transmit the character as absent. Spot *c* (fig. 27) is always a round area of variable size and it is always free, never fusing

FIG. 27.—Showing absence of variation in directions and difference in size found in spot *c* in *L. diversa.*

with any other center; even in area it is fully as conservative as it is in shape. In the former its greatest change is a condition having an irregular outline. Quantitative determinations made of the area at El Borrego and Sierra de Escamela, near Orizaba, Mexico, and at El Riego, near Tehuacan, Puebla, Mexico, for its variety *rugosa,* show the constant differences and shape of polygon, represented in figure 28. That their value is of no moment is shown by their lack of permanency; when stocks are taken to the uniform condition of the laboratory at Chicago at once all assume the same condition. In this example the differences are, as far as determined, merely unfixed habitudinal somatic fluctuations in quantity of pigment due to external conditions acting upon growth conditions.

Examinations of the other species that have formed the material basis of this portion of the investigation with reference to these simplest characters show the same kind of data and the same general results as those set forth. All of the species in the group have been gone over in this way and none have given results in any wise different from those stated. Only in detail, which is of no general interest, do the sets of observations differ. Spot *c* has been most frequently presented in this account, because of all the pronotal characters it is least liable to fusions and least variable. In all instances this "simplest character" shows heterogeneity which, by definition, may be made fluctuating or mutative, quantitative or qualitative, as one may wish. It is shown that the simplest characters differ in amounts when measured, change in different directions, and enter into diverse combinations. They are always delimited, are apparently never undelimited, and may or may not respond to heterogenetic causes in definite or indefinite reactions, so that these trivial characters react individually in much the same way that individuals and species do. To some this may mean that the individual as a whole so responds, and the character reacts in the same way because correlated with the whole. To some extent this is true, but not entirely so, because these areas have a specificity of behavior and an individuality of reaction and stability that is astounding.

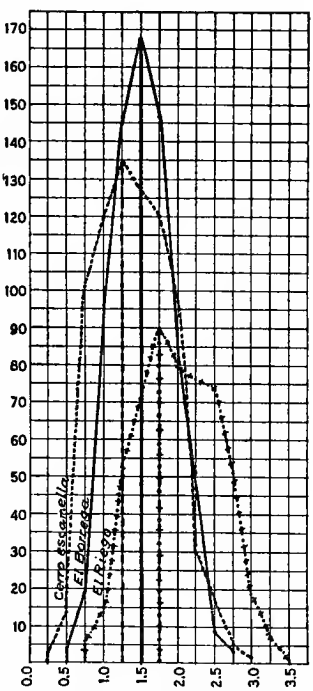


FIG. 28.—Statistical treatment of values of left spot *c* in *L. diversa* and its variety *rugosa* at El Borrego near Orizaba, Sierra de Escamela, and El Riego.

In *L. undecimlineata,* in the same locations, *c* is often wanting in a low percentage of the population, and from these individuals a race in which *c* never appears can be easily created. Of interest, therefore, is the experience of crossing one of these races (biotype) based upon a minute and trivial character with *L. diversa* or *L. signaticollis,* where *c* is never absent, and then to discover that F_2 extractives of *signaticollis* with *c* absent are obtained, and from them arise pure biotype lines differentiated by this character. The presence and absence relation is to be conceived of in this instance as applying only to the one area *c,* but equally precise behaviors have been observed in *b, d,* and in the absence of the $a'+b'+\begin{matrix}am\\+\\pm\end{matrix}+a+b$ group. The contrasting characters, minute as they are, behave in the same precise manner as do larger and more generally distributed characters. This can only mean that somehow in the gametes there are exact and detailed conditionings of this character (spot *c*), and that in crossing there is a precise recombination of the germinal agencies, such as to introduce, where it was not before, a condition of the character found in another species. While it may well be true that whatever does produce the new condition in the *c*-less extracted *signaticollis* of F_2 may be tied to or be a property of the whole, there still remains the grave difficulty of accounting for so exact and trivial a mani-

festation of the "general property of the whole" and always in the same place. Moreover, *c* does not come back into such a strain except as it is put back by the reactions of interchange of gametic factors between a *c*-less race and a race that has *c*, which may be either another homobiotypic strain or any heterobiotype that has *c* present.

Not for a moment is *c* or *b* or *d* or any other area to be thought of as a single thing or entity. It is absolutely not a unit-character in any sense. The pigment of the spot is the product of a black determiner and a color-factor; the pattern rests upon two distinct sets of agents—a general pattern-factor of the pronotum and the pattern determiner of the particular area, and this pattern determiner is always, except in pathological examples, precisely localized in the pronotum, but from this fixed position in space it is able to, and does, form different combinations by movements mutual between it and nearby areas. These combinations are always specific for the species, can be fixed and isolated in biotypes, and are alternative in crosses of such biotypes. For example, *L. diversa, angustovittata,* and *rugosa* have no directions of fusion; *signaticollis* forms *c*+*b* and *c*+*e ant.*, but never both at the same time in the same individual. *L. undecimlineata* and *panamensis* may have *c* wanting or form *c*+*e ant.*, *c*+*d*, or *c*+*b* combinations. *L. multitæniata* shows the greatest number of combinations, *oblongata* only *c*+*e ant.*, *decemlineata c*+*e ant.* and no other, while *juncta, texana, tumamoca,* and *defecta* have not thus far shown in nature or in cultures any fusions or heritable directions of variation. This behavior has a strong analogy to the condition of many molecules or radicles where there are one or more free bonds at times, at others with no unsatisfied bonds. It is not for a moment to be supposed that the reactions shown are such; only the type of action seems to have something in common in the effort of the localized mass of matter to place itself in the system with all bonds satisfied and no free bonds unsatisfied, which at any moment may become a possible entrance-point for incident dissociative forces.

These simplest characters, while not capable of disruption into lesser areas or characters, are each the visible product of the interaction of the factors and determiners above described. These are the common normal agents most concerned in such areas. There are others present, active, and important, which need not be considered here. All react to produce the character "simplest" observed, and the observed product varies in space, volume, direction of combination, quantity of pigment produced, and area occupied. Variation in these characters is not entirely of any one aspect, but is quantitative or qualitative, continuous or discontinuous, depending upon the aspect, method and point of view, and responds in delimited changes which may be definite or indefinite in the action of the observed population.

There are very many simplest color-characters in the adult that are precisely like those discussed. The head, abdominal segments, legs, and wings present color-marks and arrangements of coloration that would be serviceable in the investigation of this problem of the nature and diversity of simplest characters. I have in the course of this work gone into the study of more than a dozen of these with greater or less completeness, but always with the end-result found in those presented, and no purpose would be served by presenting a larger array at this place. It might be asserted that the instances chosen and presented are

special cases, of a highly specialized pattern, and are through "organic correlation" bound together, so that all show essentially the same action, even though they are diverse; and in order to meet this situation as well as one can, I have investigated simplest characters in the young, also structural and physiological characters.

IN THE YOUNG.

The young stages of these animals possess many characteristics that might be made the basis of studies of simplest characters. A good example of a simplest character in the larva are the spiracula spots arranged on the pleuræ in a single longitudinal row surrounding the openings of the spiracles. In all the chrysomelid larvæ that I have seen this row of spots is always, if present, found as single indivisible pigmented areas about the spiracular openings. They are never, as far as I have been able to observe in nature or produce in experiment, broken into two or more elemental areas or components of any sort, and as far as evidence is available they are simplest characters arranged in segmental series upon the abdominal segments. They may be absent or they may be removed by experimental means, especially by combined selection and environmental influences; and in this instance the presence of spots is dominant over the absence of them in races created by this means. Never is there any trace of a division into elements, so that they are to be regarded as simplest characters. In the majority of the species in this genus the spots are small, rounded, rather uniform in size, presenting little in the way of variability in exposed area on corresponding segments in a series of several hundred individuals. Reference to the figures given in this work at a different place for divers purposes will show that the character in question is extremely uniform for all, and that there is little range in size or shape in the segmental series or in the genus as a whole. Long ago, in a fit of biometric enthusiasm, I measured the spots from camera outlines made from 100 larvæ of *L. decemlineata.* Those from corresponding segments were seriated, and the whole series showed only the expected monotonous array of uniform steep-sided curves, all much alike in limits, identical in form, and only significant of uselessly expended time and energy.

When one searches for directions of combination with other spots, there too the quest is not productive of any certain results in the larger part of the forms examined. In *L. diversa* and *L. signaticollis,* however, there exists precise and distinct lines of fusion, and once one knows the position of these, it is recognized that the rather squared form of the dorsal edge of the spots that is often present has a meaning. In the two species mentioned, the top of the spot is squared, and from the anterior and posterior sides thereof there are locations and directions of pigment production medianward and that may unite with corresponding lateralward developments of the lateral tergal spots, fusing to give a U-shaped spot with a heavy base and the arms reaching dorsalward towards the middle tergals, with which fusion is not infrequently effected. In figure 29 I have shown some of the conditions in this spot that are found in *L. signaticollis.* In other species there is shown only an aborted attempt to develop pigment in these locations and directions.

In *L. undecimlineata* even this aborted tendency is absent; the spot is nearly round, and when crosses are made of these two species some interesting results

are produced in the larvæ. In the F_2 extracted *signaticollis* types there is a regular proportion of the fraternity in which the normal type of spots has been replaced by that from *undecimlineata,* giving a yellow larva with the usual number of black tergal markings, but without the characteristic fusions. An extracted race of this sort does not have the tendency to produce the directions of fusions that the normal species does; the typical spiracular spot of

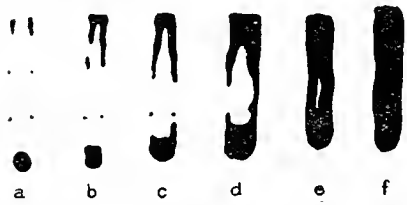


FIG. 29.—Diagram showing centers of coloration on the abdominal segments in the larval stages of *L. diversa* and *L. signaticollis,* and directions in which fusion between these elements may take place.

signaticollis has been replaced by an equally specific condition from another species by the reaction in crossing. In figure 30 I have shown these two types of larvæ, which are different enough in pattern so that anyone can at once distinguish the two. When two such biotypic races of *signaticollis* are crossed typical Mendelian arrays are always produced in F_2, showing the remarkably exact and specific nature of the productive factors and determiners of these

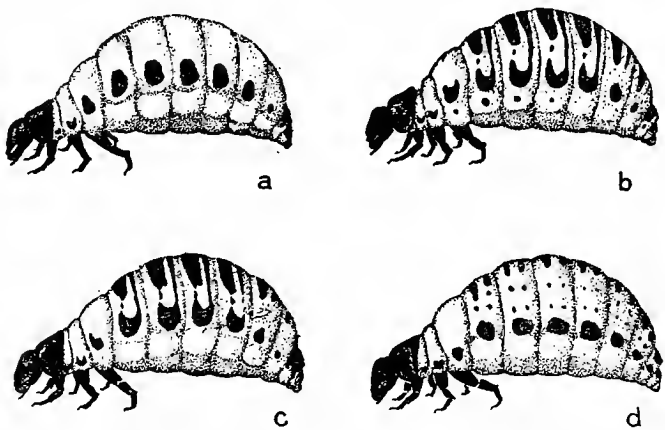


FIG. 30.—Showing pattern in third larval stage of *L. undecimlineata* and in *L. signaticollis* and effects produced in the larvæ by the crossing of these two, which results in the production of extracted races of *signaticollis* in F_2 having larvæ of two distinct types in the third stage; those like the original parent stock (C), and those intermediate between the two parental stock (B), in that in the latter extracted race the different elements never fuse, but remain separated as a stable characteristic, so that the amount of pigment presenting this stable race (B) is intermediate between the conditions represented in the two parents.

trivial, nonutile characters. There are clearly present and operative in this instance the general color-factor for black pigment (*Cm*), the black pigment producer (*Bm*), the general body-pattern factor (*PIII*), and a specific spiracular pattern-determiner (*Sp*) that are intimately concerned in the production of these spots, which are absent if one of these essential elements in its array of producers is absent. If either *Cm* or *Bm* is absent, no color is pro-

duced in the location; likewise if (*PIII*) is absent no general localization of color occurs, a diffuse distribution with no specific spots resulting, while *Sp* is necessary to the series to give specific form to the area in question.

It is easy to show by present methods that there are four necessary active agencies at work in the production of this series of spots, and while it is not known what the nature of the agent is that produces the action in any instance, the accuracy of operation and the certainty of the result leaves not the slightest doubt that these agents are at work in the production of the spots in question.

I have tried repeatedly to obtain, from the study of the spots in the larvæ of these organisms, evidence that there were various categories of "variation" in the differences shown, but I find myself unable to decide which differences are quantitative or qualitative, which in line or multifarious, unless I ignore obvious mistreatment of the data and use unproven assumptions as true. The larvæ afford numerous simplest color characters, and can with adequate tests show in *L. decemlineata* or any other species that the character presents differences in quantity, arrangement, relations in space, quality and specificity of manifestation; in short, it presents the array of attributes, qualities, and conditions that any resultant of interacting substances and forces do, and these are heterogeneous in their manifestations.

In the effort to understand the meaning of heterogeneity in the characters of organisms, using these beetles as materials for study, I have made such use of these simplest color-characters in the effort to understand the significance of the characters themselves and the nature of their production and significance of their differences. There has been the problem to satisfy myself concerning the two categories of "variation," as defined by De Vries, to discover if the distinctions made are valid, because if so they are fundamental for further investigations of evolution problems. Thanks to neo-Mendelism, the nature of characters has received a searching study and analysis as never before, and while incompleteness is apparent in neo-Mendelian work, it has shown clearly that there are in the production of the characters of organisms agents, carriers of nothing in particular but the properties of their own substance and capacity of action, but which, when present in the mechanical system of the organism, produce the concrete product and interactions in exact and predictable results.

I have tried to give the De Vriesian criterions with regard to the diversity in organisms an adequate and fair test in this material in its color, as well as in other characters, as illustrative of a great series of "organic characters." If it be assumed that pigment is the character, which is contrary to fact, and if it be further assumed that the amount of it present represented the homogeneous result in the population of uniform causes, which is also contrary to fact and if it be further assumed that the measurement of the area of pigmented surface is an accurate measurement of the amount present, which is also contrary to fact, one is ready to begin the routine statistical study of the character and arrive at "exact mathematical proofs" and demonstrate some "fundamental facts of biology." De Vries took at their advertised face value the supposed accurate results of the biometricians, and therein lies a serious defect in his treatment of the problems of the origin and meaning of "variation" in organisms. No statistician has taken care to determine that his measured material is homogeneous, or that his measured "characters" are single characters or dimensions

of single characters, and the neglect of these alone is sufficient to invalidate their findings. The added difficulty that these fluctuations were not capable of unlimited accumulation through quantitative selection seemed an insuperable obstacle to De Vries, and while there is no doubt of the general correctness of this limitation and the frequent regressions to the mean, both limitations and regressions are due to other agencies than those considered by De Vries.

ANALYSIS OF HETEROGENEITY OF SOME SIMPLEST STRUCTURAL CHARACTERS.

With structural characters it is necessary to apply the same criteria for their determination as in the color-characters, in that they must be the indivisible resultant of the interaction of a single constellation of interacting agencies, the absence of any one of which results in the non-production of the character, in precisely the same manner that the absence of an agent in a complex physical system results in the non-production of the proper product of the operation of the mechanism.

IN THE ADULT.

Prime favorites with the statistician and the taxonomist are such "structural" features of the organism as the dimensions of parts, length and breadth or indices, form, *i. e.*, elongate, robust, depressed, general aspect of the surface, rugose, glabrous, punctate, and so on without limit. These characterizations of the appearance and form of the object serve well in the operations of the taxonomist to distinguish the different species, but finer analysis is needed for the investigation of the problems of the origin and meaning of diversity in organisms, and especially in the effort to untangle some of the conflicting statements regarding the problems of heterogeneity.

In the case of structural characters, the same set of questions need to be asked and investigated with care as in the case of color-characters, and the same exact requirement needs to be followed so that homogeneous states shall be investigated. Both must be the indivisible product of a group of interacting agencies, in which the absence of any member is followed by the non-production of the character in question. It is necessary to deal with simplest structural characters, as the basis of the analysis.

In the organism with which I have been working there is an unusual dearth of spines, scales, or other ornamental characters of a "structural" type, only the integumentary punctations being present. It has, therefore, been necessary to use more important portions in the study of the characters of this class, although it is by no means as easy to obtain simplest conditions as in color-characters.

Body-weight.—One of the first characters investigated was the variability in the body-weight of the population, which, obviously, is the product of a large number of contributing factors in the population and in the individual. A deal of time was spent in 1899, 1900, 1901, and 1902 in the statistical study of body-weight in *L. decemlineata* Say. Both the live weights and the dry weights were obtained, but the outcome of the study was the demonstration that the method and the material could give nothing of value. I was using materials from nature, and these were obviously of unlike composition genetically, of dissimilar

experiences in their ontogeny, and came into my hands for study from the field in quite heterogeneous states of nourishment, age, and reproductive activity.

It was thought that the " character " might be of some interest in the study of " variation " phenomena, and in that similar weight determinations had been used in investigations, there was no *a priori* reason why the same might not be of interest in this material. This first study of the weights as a structural character was so unsatisfactory that I did not follow it further at that time. There were too many obvious sources of error in the set of observations, too little homogeneity of materials or determinations, even though the statistical method did blend them beautifully in the end; so that the attempt was abandoned from the point of view of materials taken in nature, even with the greatest care as to location, generation, and conditions of life and stage of development.

In 1901, 1902, and 1903 I made a different plan of attack upon the problem of weight as a " unit-character," this time breeding from single pairs, obtaining the weight of the progeny at the onset of the breeding-period, and in the next generation breeding only from the individuals that had the same weight as the parents, and so on. Effort was made to have the material as uniformly nourished at all times as possible, to give it all the food it would eat, and to weigh at a fully nourished state at the onset of reproduction activities. Other conditions were not controlled or measured and no attention was paid to them, excepting to make them as nearly normal as possible, so as to give the best growing-conditions for each group and produce homogeneous materials. These measurements were made in a series of experiments to modify the size of this species by selection, and the measurements here recorded were a part of the measurements made in the course of that study, and serve well in this place to illustrate what one really finds in the variation of characters of this class.

In the population which I was using for this work, which came from eastern Massachusetts, I weighed both males and females, and the means of the weight at breeding were males 7.5 to females 10. In figure 31 is given the results of the weighings of the males in four generations. At the start three sets of weights were mated—light, average, and heavy—the male being used as the basis of the determinations. They were mated with females whose weight was to the males' weight as 10 : 7.5., thus preserving " statistical equality " in the matings. From the lightest condition mated (*a*) a line of descent was carried out as shown in figure 31. In the first generation are shown the matings from the different light, average, and heavy pairs, with the male weights alone given, showing that the three conditions of weight gave about the same conditions in the array of the weights in the progeny. In figure 31 are given the results in the line of descent from the light condition (*A*) for four generations. The materials subtended certain weight values, in terms of more and less, and in consequence were at once arranged in a line distribution of plus and minus; but the array of values showed nothing of any interest, save the shortcomings of statistics and their inability to analyze the condition in the material under investigation. I was interested in this set of determinations for the reason that so many similar determinations had been made with far less care than I had employed, and not infrequently far-reaching conclusions had been drawn, the reason for which I could not discover. The general result is simply that statistically determined weight is not a simple character of the organism, although not infrequently so considered.

Corresponding lines from the average and the large weights were also carried, but are not shown in the figure because they would only complicate it with the larger number of curves and add nothing to the results in that they were precisely similar to those shown in the *A* series.

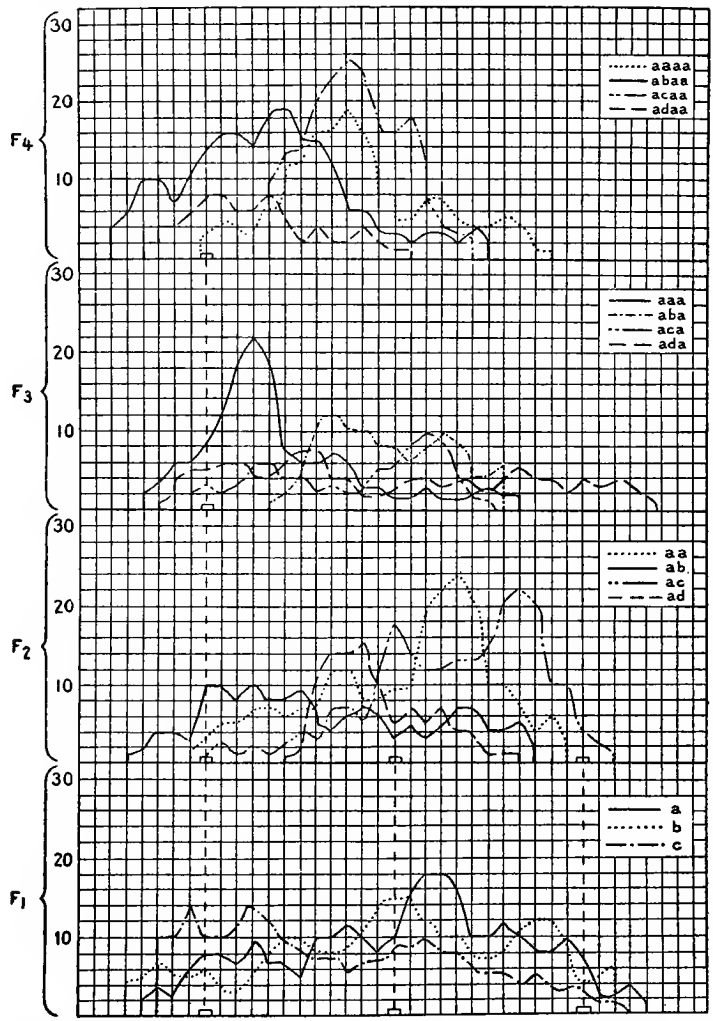


FIG. 31.—Schematic representation of results obtained in the attempt to breed weight as a single character, showing distinctly that it was not possible to purify this material with respect to weight and reduce it to uniform pure lines.

In the years 1907, 1908, and 1909 I made a similar set of determinations upon races of *L. signaticollis* from the Rancho Basosco location, these being bred out in line cultures and selected on the basis of weight. The general result was the same as in the two preceding instances, and there were no results but a lot of values subtended by the animals in question and having no certain relation to the organism.

Much use has been made of the instances of improvement made in the domesticated organisms by selection of "quantitative variations," and all are thoroughly familiar by this time with the extensive use that has been made of these instances by De Vries in the formulation of the mutation theory, and by Johannsen and his followers in the development of the pure-line hypothesis. In all of these instances it is assumed that there is "quantitative variation"; but is it proven that there is such? Has it not been assumed that there are quantitative variations solely on the basis of the inaccurate findings of the statisticians? In what instance has a biometric worker really determined the nature and homogeneity of his material before he began to investigate it through statistical methods? Recall, for example, the many determinations made by Pearson, Galton, and others of the same school in the human species, with entire lack of homogeneity of parentage, race, conditions of growth, present conditions of life, and other factors that may enter into the production of the individual. It is results from examinations of this sort that have led to the existence of "quantitative fluctuating variations" in the literature of the last two decades, and lead primarily to the creation of the situation in this field that arose with the mutation theory.

The history of the sugar beet has been again and again used as an example of the existence of "quantitative" or "fluctuating variations"; but is the determination of the saccharine content of the beet the character, and does it represent this group of substances as they exist in the beet? In this instance a heterogeneous group of compounds, the saccharine content, has been removed from the organism, is determined in percentages of total weight of the body from which it came, but the extracted product may have many mono- and di-saccharides in it, but is rated as "one character," and no attention is paid to its diversity in composition and the fact that it is an *extracted product* and not a character *in* the organism. As to the materials in the organism, there is relatively little information, but that available indicates complex relations, the product of divers processes at work. For the practical purposes of the sugar industry the amount of "sucrose" that can be extracted is important, and the development of strains that produce large extractable quantities is commercially desirable, but extractable amount measured quantitatively is hardly a determination of the character as it exists in the organism. There is nothing in the work to indicate whether the differences in the saccharine content in the sugar beets are fluctuations in quantity or not. In the mono-saccharides and di-saccharides present there is remarkable specificity of the different substances in composition, action, and relations in the organism, and differences of most numerous sorts may well exist without being discovered or even considered by the statistical treatment of the "character."

In this instance saccharine content is not the only "character" that has been considered; form of the root, woody content, leaf character, and others have come under the observation and manipulation of the beet grower, have been purified, and races derived having the desired commercially valuable characteristics. In this practical work the operators, misled by the selection idea and the complacent uncritical methods of the statisticians, applied "measures" to the characters, which although fairly useful to a limited extent for the practical purposes of industry, did not and can not express or indicate the real relations of these "characters" in the plants. These are most complex, and differences of all

sorts are presented which can not be separated into rigorously limited classes of quantitative and qualitative "variations." In no instance in operations of this kind have the workers been dealing with simplest characters, or even with unit-characters in the sense of De Vries, but usually with an undetermined complex, capable of subtending some mathematical value, and the mathematical value has been regarded as a true representation of the character in question. It is largely this sort of thing that has led to the establishment of the idea of quantitative variations by De Vries as distinct from qualitative.

The fact that the continued "selection" of these characters does not result in the production of unlimited divergence of the character, which is a difficulty in the minds of many, is not really an element in the present problem. It is quite distinct, and it may well be asked whether any kind of character is capable of unlimited "improvement" by any method of transmutation whatsoever. It seems that the very nature of natural substance would indicate that there are limits to combinations, to arrangements, and to operations, and the limitation of "selective action" by bounds that are not passable is not surprising.

The "difficulty" that has entered so largely into the discussion of the demerits of fluctuations as an effective element in the modification of organisms, "that the products of fluctuations can not be fixed in position," does not seem at present to offer any difficulty. Fixity in position is simply stability of composition, method of reaction, or both, and homogeneity of organization; and it is precisely this that the selectionists have not taken into their considerations and operations. No effort has been made to obtain materials or characters that were single and pure, and a heterogeneous mess extractive like saccharine content, that can be measured in mathematical values, is the limit of their ability to analyze either method or product or character. I shall have occasion to deal with this question further when I come to consider some of the experiments in "selective improvement," but the main point that I wish to make at this place is the uncritical use of the biometrical efforts in the last two decades, as an example of an inefficient type of investigating the origin of heterogeneity, in which the differences were assumed to be mere differences in the amount of something present. Without reference to the correctness of some of the conclusions regarding the lack of effective transmutation produced by these differences, and all of the consequences that follow therefrom, it is becoming increasingly clear that diversity is not one of amount present, but that the origin and nature of diversity is best stated as integrated products of associations of productive agencies whose presence is necessary for the production of the result and in whose array any break results in nonproduction of the usual manifestation. In some instances it might be possible to express this integration in single terms of measure, but these can never express the really complex nature of the result, in which there are always present relations of position in space, interaction with adjacent materials, the intricate series of antecedent series of events that preceded the end-result, with the constant play of many incident agencies from within and without the mass of the system, and to regard this complex series as a mere quantitative swing of the amount present and as expressing in measure the amount of units subtended on the biometricians' meter-stick, is to blind ourselves to some of the most obvious facts of nature, and to indulge in the entirely useless and false creation of categories and definitions that are not in any way useful in investigation or expressive of certainly determined facts of nature.

In 1906 I gave the results obtained in the study of the size of *L. decemlineata* as determined statistically in two locations. In these determinations more than the usual care was taken to have the materials of uniform composition, as regards location, generation, and the possible presence of material of other generations. The result expressed at that time in quantity showed only the customary array in a polygon of distribution without significance. This I followed in the population at West Bridgewater, Massachusetts, through 16 generations, and at Chicago I followed the same investigation through 8 generations, with the same results at both places. I was interested in doing this because of its bearing upon many problems of evolution and transmutation. The net result of this and similar examinations made in the same way was to show that the biometric method was seriously invalidated by the failure to consider the action of place variation, and the further false assumption that the materials collected in nature are homogeneous, even when collected in one location of the same generation and at the same time. It was early evident in my determinations that the material was heterogeneous, and hopelessly so, as far as any effort to simplify it by biometric means was concerned. I kept up the effort, using all of the precautions possible with the collections made in nature, both with our native species and those of the tropics, with the idea of giving this " quantitative variation idea " the fullest and fairest test that I could, and for this express purpose I kept in the background other ideas and methods of investigation.

" Graduated variates," as determined by measurement of stature or other dimensions, have been the basis of much " quantitative variation " literature and arguments, and the stature, or longest diameter of the mass of the system, is often assumed to be a unit. Neo-Mendelian investigations have shown beyond doubt that in many instances " stature " is a specific property of a mass and does not blend into intermediate masses in fixed combinations, but segregates out of any mixtures into which it enters. The experiences with plants of different statures, of different sizes of animal forms that segregate out, and of which an ever-increasing array of instances is being discovered by enthusiastic workers, leads one to be highly doubtful of the " blended " nature of these characteristics in any organism and of the existence of a long series of " graduated variates " therein.

Without any exception, I believe, everyone has accepted the idea that at some point in the series there was a certainty of there being " graduated variates " that were " quantitative fluctuations " in the " amount " of the character present. The biometricians, starting with heterogeneous unknown material, measured anything that was convenient and further complicated it with the application of their treatment of the data, while the De Vriesians, starting with refined material in pedigree cultures, attained an exactness of analysis and refinement of results quite beyond the ken of the former school. The unit-character, however, was not destined to long survive the searching analysis given " characters," and gave place to the conceptions of Johannsen and his followers. All have retained the conception of the existence of quantitative fluctuations. In reality the problem has not been analyzed; rather with the recognition of more and more refined units of operation in the investigation, and the establishment of the reality of these, the fundamental propositions as regards the nature of the diversity presented by the unit has been assumed and not investigated.

Significant as are the investigations of Johannsen with the genotypes of plants and of Jennings in the clones found in *Paramœcium* with respect to these problems, there still remains in both the idea of quantitative as opposed to qualitative "variations" bearing much the same relation to one another that they do in the original De Vriesian statement.

Since most of us can not admit the possibility of any "specific biological principle," or vitalistic content in the phenomena of our science, it follows that the essential processes in one of the most evident phenomena of organism, the rôle of heterogeneity in transmutation and evolution must be capable of expression in terms and in principles that were equally applicable to both organized and non-organized bodies; and the same principles of investigation should, with differences in the means employed, be applicable to and give consistent results in both sets of material aggregations. This working hypothesis may be formulated as follows:

(1) *The recognition* that only physical principles are operative in the phenomena classed as organic—a principle that is in entire accord with all of the investigated and analyzed organic phenomena.

(2) *The recognition* that the characteristics of organisms are of the same origin and kind as in non-organized bodies and are *specific properties, attributes,* and *conditions,* and have in organisms precisely the same origin and relation to the whole that they do in non-organized masses.

(3) *The hypothesis* that heterogeneity is the product of the composition of the material by the conditions of the medium during the production of the combination and influenced by the degree of purity of the combining materials and presence of uncombined substances, which may influence the end-result in the manifestation of specific properties and attributes, and be the active causes of heterogeneity. These operations and results, when fully analyzed, will be found to be but expressions of established principles of physical science.

An analysis of heterogeneity must, therefore, be made on the following scheme:

(1) An analysis of the constitution of the substance under standard constant conditions of observation.

(2) The determination of changes in the medium upon constancy of the substance.

(3) The determination of loosely combined or uncombined "impurities" in the organic system, and the rôle which they play in the production of fluctuations in the characters of the material.

(4) The isolation of non-fluctuating lines and study of the action of changed conditions and introduced factors in the system, to determine the nature of the so-called "fluctuations."

The defect in biology is that homogeneity has been assumed without being tested at all until recent years, and then inadequately.

I began in 1904 an inquiry into this aspect of the "variation" problem, and for this purpose I took the most variable and complex member of the group, *L. multitæniata* Stål, which I was certain would give me no end of trouble and present about all the complications that one would expect to find in any organized body. In this I was not deceived.

It was recognized at the start that it was possible to obtain with the methods in use an abundance of data, which could, with ingenuity, be arranged into

curves of "quantitative variations" and those that stood apart and were to be characterized as "qualitative." These same data would, with proper treatment, give an imposing array of constants of no value or meaning, as far as the analysis of the origin and meaning of the very evident heterogeneity was concerned, and having already obtained an abundance of this sort of information regarding these materials, at the onset of this study I discarded entirely the method and attacked the problem from the point of view of genetic composition and the effects of conditions in the medium.

I have shown in Chapter II something of the natural complexity of this material, of the forms that exist in nature and of the wide range in many of its attributes. I was forced into this study by the attitude of De Vries in the mutation theory and the position of Johannsen in his 1903 paper, and by all of the current writers at that time, who as a body held to the two categories of "variation." Since that time the progress of neo-Mendelism has produced many changes in the viewpoint, with closer and closer approach to the conception of heterogeneity conceived of in purely physical terms. It is clear that De Vries thought of mutations and fluctuations as distinct, and that the latter were either of germinal or somatic origin, distinguished by the fact of their inheritance, and if so, capable of accumulation up to certain limits. East in 1911 has in common with many others limited the term "fluctuation" and placed the germinal variations as follows: "A fluctuation is a non-inherited variation produced upon the soma by environmental conditions, while the inherited variation, the mutation if you will, is any variation quantitative or qualitative, that is germinal in character." It follows from this that any diversity in the observed series that is not inherited is then the product of the environment upon the organism during its ontogeny. Since it was non-visible in the following generations, it follows that it was absent as a part of the germinal complex, which may or may not be true, and the further assumption that since it was not inherited as per test it was the product of incident external conditions upon the organisms during its growth. This is characteristic reasoning from effect to cause, and then broadly applying the result as a criterion to separate the kinds of heterogeneity present.

FORM-INDEX.

For the study of form-index I took a body-index as representing a measurable character of the entire mass, and comparable to stature, tall and short in some of the neo-Mendelian operations, and roughly equivalent to the same index that might be derived from inorganic masses. These animals, like other organisms, are during ontogeny changeable in some of the measurements of the body that shift with the state of nutrition, reproduction, and age, so that I used only measurements that were free from these sources of obvious error. In figure 2 I have shown the measurements taken, the distance between parallels passing through the anal apex and the anterior margin of the elytron (1), the same measurement between the parallels passing through the anterior and posterior margins of the pronotum (2), and the corresponding measurement of the epicranium (3). These are not influenced by the conditions of nutrition or the changes in form incident to breeding. They were added to give the length or longest measure of the mass; this was divided by the greatest breadth (4), taken as the greatest distance between the lateral margins of the pronotum, and this

gave body-index. The breadth of the elytra would have been useful, but too much difficulty was encountered in eliminating obvious sources of error in the measurement. This index is a good indicator of the general "body-forms" so often useful in taxonomic work and a real characteristic of the mass. On plate 26 are shown some of the different forms of body which this species exhibits, and in the main can be grouped into three assemblages, of which the *multilineata* form represents one extreme, some of the *melanothorax* types the other, and the typical *multitæniata* the intermediate. Any one of these types is "variable" in its form as found in nature and even in cultures that have been purified with reference to the color and pattern differentials. My problem was to purify one of these forms if possible, and then analyze or produce in it "variations," in the hope of being able to gain some clue to their causation and significance. I took the *multilineata* type as the start in the problem as being the simplest, and the problem was to obtain a constant and pure *multilineata* type.

In the years 1904, 1905, and 1906 many preliminary tests were made, so that at the end of 1906 I had 6 lines of this material that were thought usable as a basis for the study. In the course of breeding experiments with this species I had found and identified three sets of factors that were productive of form and form changes in the species *L. multitæniata,* and I had been able to prove the reality of these by the repeated transference of them to the species *L. decemlineata* and *L. oblongata,* replacing in the normal form the "body-form factors" from the strains of *L. multitæniata.* The materials which I had at the end of 1906 were 6 races with the form-factors so purified by being repeatedly extracted and purified in genetic operations that the strain was highly constant in both size and shape, ranging well within the index limits of 2.500 and 3.000, and there also had been an effort to reduce the complications in color and other characters as far as was possible, so as to give a homogeneous-acting biotypic form. The strains were now in the condition that would have admitted being characterized as highly refined "biotypes" showing only the usual expected "fluctuation" about an average value.

In 1907 I carried 5 of these through 2 breeding-periods, or 4 generations in all, and of the 5 all showed the same results in that they were constant to the type of the line, with the usual range of divergences in the character chosen for the examination. All would have been characterized as constant stocks, and the problem became, what was the cause of the range presented by the index?

The sixth line of the original series at the beginning of 1907 was composed of 62 males and 66 females that were the progeny of a single pair of parents and emerged from the hibernation in the winter of 1906-07, and were an extremely uniform lot of individuals. These were divided at random into 4 lots of 15 each, and within each of these lots 5 pairs were mated at random, and these bred out through 2 generations in March, April, and May 1907, and entered hibernation in the early days of June. The progeny from these matings were uniform and like those from the 5 main stocks reared during the same time and under the same conditions; 17 of the pairs gave progeny and the range in the index of these, as well as the same character in one of the 5 "stock" lines, is shown in table 28 for the first generation of 1907 and in table 29 for the second generation of 1907.

All that the tables show is that the 5 stocks and the 17 pairs were like in their index and with ranges of the individual fraternities that in the main were similar. In all respects a "pure" biotypic strain had been isolated in this complex species, showing the "customary fluctuations." What was the nature of the fluctuations?

TABLE 28.

No. of mating.	Parents, index. M.	Parents, index. F.	Progeny. Fraternity No.	Sex.	Range of index. 2.500–2.549	2.550–2.599	2.600–2.649	2.650–2.699	2.700–2.749	2.750–2.799	2.800–2.849	2.850–2.899	2.900–2.949	2.950–2.999	3.000–3.049	Totals.	
1. g P.	2.757	2.558	1. g I	M.	...	1	2	6	9	15	7	2	1	...	...	43	87
				F.	...	1	1	5	11	17	8	1	...	...	...	44	
2. g P.	2.741	2.962	2. g I	M.	1	1	1	7	12	18	14	6	3	1	...	64	118
				F.	1	...	1	5	9	16	11	4	2	...	...	49	
3. g P.	2.569	2.754	3. g I	M.	...	...	1	2	6	9	5	3	1	...	...	27	61
				F.	...	1	1	4	7	10	6	3	1	1	...	34	
4. g P.	2.701	2.754	4 g I	M.	...	...	...	2	5	11	4	1	...	...	...	23	53
				F.	...	...	...	1	7	12	8	3	1	...	...	30	
5. g P.	2.564	2.776	5. g I	M.	...	...	2	6	11	9	4	1	...	...	...	33	69
				F.	...	...	1	5	9	14	5	1	1	...	...	36	
6. g P.	2.778	2.983	6. g I	M.	1	1	2	3	7	12	9	4	2	1	...	42	81
				F.	...	1	2	4	6	14	8	3	1	...	...	39	
7. g P.	2.731	2.728	7 g I	M.	1	1	4	8	16	5	3	...	...	...	...	38	84
				F.	...	2	5	9	17	7	4	1	...	...	...	46	
8. g P.	2.521	2.725	8. g I	M.	...	...	1	4	7	3	1	...	...	...	...	16	37
				F.	...	1	2	4	8	5	1	...	...	...	...	21	
9. g P.	2.631	2.940	9. g I	M.	2	4	3	5	8	1	1	2	1	...	...	27	61
				F.	1	5	11	7	7	1	1	1	...	...	...	34	
10. g P.	2.700	2.799	10. g I	M.	...	...	1	1	4	9	14	12	7	1	...	49	109
				F.	...	1	2	5	10	17	11	7	5	2	...	60	
11. g P.	2.754	2.961	11. g I	M.	...	1	1	1	5	7	4	1	1	...	...	21	32
				F.	...	...	1	...	1	4	3	1	...	1	...	11	
12. g P.	2.689	2.892	12. g I	M.	...	1	4	7	3	1	...	...	1	...	...	17	34
				F.	...	...	1	2	9	4	...	1	...	...	...	17	
13. g P.	2.781	2.760	13. g I	M.	...	1	...	1	1	2	1	1	...	...	...	7	19
				F.	...	1	...	1	3	4	2	...	1	...	...	12	
14. g P.	2.965	2.769	14. g I	M.	...	1	1	3	5	7	4	1	1	1	...	24	55
				F.	1	...	1	3	6	9	7	2	2	1	...	31	
15. g P.	2.581	2.794	15. g I	M.	...	...	1	1	4	5	3	1	1	1	...	17	34
				F.	...	...	1	1	4	7	2	1	1	...	...	17	
16. g P.	2.913	2.622	1C. g I	M.	...	...	...	1	3	14	9	15	7	1	...	40	100
				F.	...	...	1	3	6	17	12	8	8	4	1	60	
17. g P.	2.600	2.810	17. g I	M.	1	4	7	11	17	9	4	1	1	...	...	55	118
				F.	...	1	5	8	13	18	11	4	1	1	1	63	
Stock 2 Mass.	2.924 2.531 2.754	2.728 2.835 2.744	M. 2. g I	M.	...	2	19	21	46	31	19	11	7	4	3	163	327
				F.	...	1	11	25	34	45	21	18	6	2	...	164	
Stock 2 Pt. A	2.634	2.911	M. 2. g I(A)	M.	...	...	1	3	5	4	1	...	...	...	...	14	37
				F.	...	1	1	4	8	7	1	1	...	...	...	23	
Stock 2 Pt. B.	2.595	2.701	M. 2. g I(B)	M.	...	...	1	3	5	9	7	6	4	1	...	35	72
				F.	...	1	1	4	7	11	8	5	1	1	...	37	
Stock 2 Pt. C.	2.781	2.811	M. 2. g I(C)	M.	...	...	1	1	1	4	2	1	1	...	...	11	34
				F.	...	1	1	2	5	9	3	1	1	...	...	23	
																Total	1617

Three possibilities existed with regard to these ranges in the index: (*a*) they may be entirely environmental in origin and show simply the plasticity of the material in its response to the conditions of the medium; or (*b*) the range may be due to "impurities" in the organism, which are present in a more or less loosely combined state, but produce divergence and a range in some of

the characteristics, as do similar conditions produce like ranges in characters of non-organized matter; and (*c*) it is possible that the range is due to many small actual differences in the constitution—"germinal variations"—which the methods used or the criteria of the index used are not able to separate out into the component groups.

TABLE 29.

No. of mating.	Parents, index.		Progeny.														
			Fraternity No.	Sex.	Range of index.											Totals.	
	M.	F.			2.500–2.549	2.550–2.599	2.600–2.649	2.650–2.699	2.700–2.749	2.750–2.799	2.800–2.849	2.850–2.899	2.900–2.949	2.950–2.999	3.000–3.049		
1. g I	2.761	2.599	1. g II	M.	...	1	1	4	9	11	8	5	1	...	...	41	87
				F.	1	1	3	7	13	10	6	4	...	1	...	46	
2. g I	2.749	2.931	2. g II	M.	2	1	5	22	31	46	30	11	4	1	1	154	313
				F.	1	3	11	19	29	45	33	10	6	2	...	159	
3. g I	2.560	2.751	3. g II	M.	...	4	14	21	27	20	16	8	3	1	...	114	193
				F.	...	2	4	5	9	17	22	14	5	1	...	79	
4. g II	2.739	2.760	4. g II	M.	1	...	6	19	40	46	31	17	8	3	1	172	275
				F.	...	5	11	24	39	13	8	2	1	...	...	103	
5. g I	2.570	2.771	5. g II	M.	1	2	1	5	8	13	17	11	5	2	...	65	146
				F.	...	7	13	16	19	14	6	5	1	...	...	81	
6. g I	2.790	2.870	6. g II	M.	...	1	2	5	9	13	8	4	1	...	...	43	102
				F.	1	2	5	6	6	17	9	7	3	1	...	59	
7. g I	2.738	2.731	7. g II	M.	1	1	3	10	21	9	6	1	1	...	...	53	114
				F.	...	1	2	5	12	24	11	4	1	1	...	61	
8. g I	2.529	2.700	8. g II	M.	...	2	5	4	8	9	10	7	5	2	...	52	101
				F.	...	2	3	8	14	11	7	3	1	...	...	49	
9. g I	2.619	2.901	9. g II	M.	...	3	8	14	19	17	9	5	3	3	...	81	157
				F.	...	...	1	6	10	21	23	16	5	3	1	76	
10. g I	2.700	2.791	10. g II	M.	...	...	...	2	6	9	15	8	4	2	...	46	95
				F.	...	2	3	7	9	15	10	1	2	...	...	49	
11. g I	2.752	2.898	11. g II	M.	...	5	5	6	8	16	11	5	1	...	...	57	111
				F.	...	4	1	4	10	16	9	6	3	1	...	54	
12. g I	2.689	2.894	12. g II	M.	...	3	3	6	12	16	11	5	1	...	...	57	113
				F.	...	4	3	5	11	19	7	4	2	...	...	56	
13. g I	2.766	2.715	13. g II	M.	...	1	1	1	2	4	3	1	1	...	...	14	38
				F.	1	1	1	3	5	7	4	1	1	...	...	24	
14. g I	2.969	2.769	14. g II	M.	...	3	6	11	29	46	31	17	5	3	...	153	315
				F.	2	5	9	14	33	47	35	14	2	1	...	162	
15. g I	2.580	2.763	15. g II	M.	...	6	17	39	51	61	42	15	3	1	...	235	423
				F.	...	3	13	40	57	47	19	6	2	1	...	188	
16. g I	2.900	2.715	16. g II	M.	...	...	2	1	2	3	5	3	3	1	1	21	52
				F.	...	3	1	5	8	4	3	4	2	1	...	31	
17. g I	2.600	2.825	17. g II	M.	1	...	3	6	13	14	11	5	3	1	...	57	118
				F.	...	3	5	12	16	11	7	3	2	2	...	61	
M. 2. g I	2.951 2.600 2.754	2.700 2.815 2.749	M. 2. g II	M.	5	14	78	117	146	122	97	46	17	8	...	650	1212
				F.	...	7	19	48	129	151	101	77	13	11	6	562	
M. 2. g I(A)	2.630	2.911	M. 2. g II(A)	M.	1	3	5	9	16	7	4	1	3	1	...	50	126
				F.	...	3	8	5	9	11	19	12	7	2	...	76	
M. 2. g I(B)	2.594	2.715	M. 2. g II(B)	M.	...	1	3	8	14	24	11	5	4	1	...	71	144
				F.	1	...	5	6	12	25	13	6	3	2	...	73	
M. 2. g I(C)	2.785	2.826	M. 2. g II(C)	M.	...	1	3	1	7	11	5	3	1	8	1	36	78
				F.	1	2	1	4	8	12	6	1	4	1	2	42	
																Total	4113

The first attack was made to determine whether the "environment" was either primarily or even partly responsible for the condition found in the range exhibited in the index. For this, therefore, I set up 12 breeding-tanks of the construction and arrangement of control shown in figure 32 (Chap. I), and arranged to give constant conditions of temperature and moisture, air-move-

ment, evaporation, and soil-moisture. The temperature was set at 21° C., the relative humidity at 75 per cent, and the air was changed once each minute, giving uniform evaporation rates.

In September 1907, I mated, under the controlled conditions, 3 pairs from pair 1 of stock 6, 2 from pair 8, 4 from pair 10, all of these taken at random in the population of each parent fraternity. The method of doing this was to separate the sexes, place each in a dish under a dark or opaque cloth, and then draw them out singly as needed for the matings, thus preventing any possible selection in the matings of the pairs within the population. These 9 pairs of random matings in different lines were placed in the tanks, and there 8 of them gave progeny. At the same time 3 mass cultures were mated at random, and consisted of 3 of each sex taken from the progeny of pairs 2, 14, and 16, and these were allowed to breed under the conditions of the experiment as were the

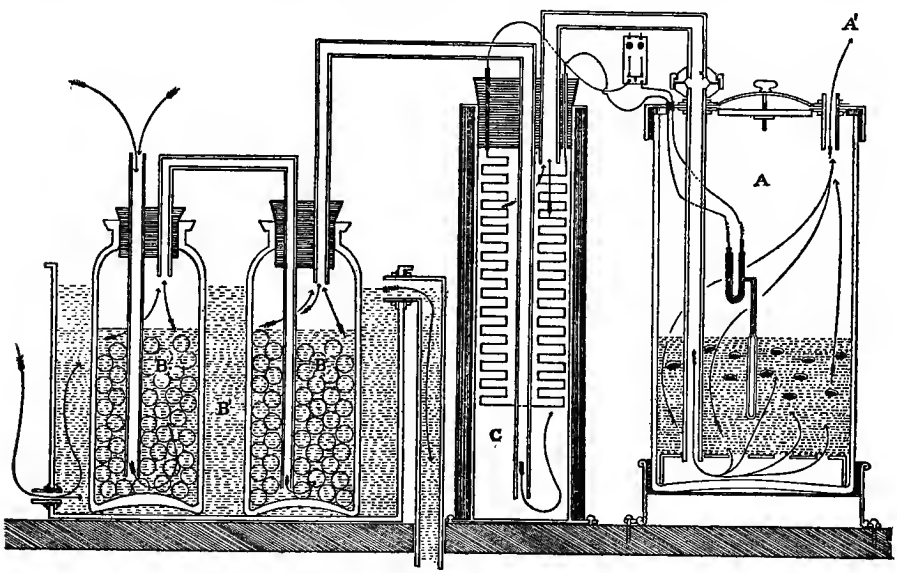


FIG. 32.—Diagrams showing different measurements taken to determine the body index in these animals. (See text.)

pairs, all giving progeny. The small size of the breeding-chambers made it necessary to grow only small populations, but each pair was allowed to breed until death; the elimination was made in the eggs and was, of course, purely a random one, and consisted of destroying about half of every batch of eggs laid.

Mated pairs and mass cultures gave normal, strong progeny in the middle of October, and presented no difference in the indices or in the range thereof in any of the populations resulting. At the end of October each line was continued by another random mating, and in the same tanks as before, and these all gave progeny, reduced in number as before, in the first half of December 1907. The resulting progeny, however, showed exactly the same ranges in the index as did the previous generation and as did the other portions of the series, namely, the progeny of the other stocks that were reared at the same time, but not under accurately controlled conditions. I was greatly surprised at this result, having expected that there would be an alteration of the range in the index in at least

some of the lines from the mere fact of growing them under uniform and constant conditions; but no such effect was found.

All of the strains hibernated from late December 1907 to the first of March 1908, and were then mated within the same lines at random and in the same set of conditions, giving a generation in April and a second one in late May and

TABLE 30.

No. of mating.	Parents, index.		Progeny.													
					Range of index.											
	M.	F.	Fraternity No.	Sex.	2.500–2.549	2.550–2.599	2.600–2.649	2.650–2.699	2.700–2.749	2.750–2.799	2.800–2.849	2.850–2.899	2.900–2.949	2.950–2.999	Totals.	
1. g II(A)	2.715	2.841	1. g III(A) ...	M.	...	1	1	2	14	8	5	1	1	1	34	69
				F.	1	3	4	4	7	15	6	2	1	...	35	
1. g II(B)	2.619	2.737	1. g III(B) ...	M.	...	1	2	4	8	3	2	1	1	1	23	52
				F.	1	...	1	7	11	5	1	2	1	...	29	
1. g II(C)	2.771	2.742	1. g III(C) ...	M.	...	...	1	4	7	14	5	1	1	...	33	73
				F.	...	1	3	7	8	12	5	1	1	1	40	
8. g II(A)	2.779	2.601	8. g III(A) ...	M.	...	...	1	1	3	5	9	4	1	2	26	49
				F.	...	1	2	1	4	8	2	3	1	1	23	
8. g II(B)	2.658	2.711	8. g III(B) ...	M.	...	1	...	1	4	7	3	2	1	1	20	39
				F.	...	1	1	2	4	6	3	1	1	...	19	
10. g II(A)	2.811	2.614	10. g III(A) ...	M.	...	1	1	2	4	8	1	1	...	1	14	30
				F.	...	1	1	3	6	1	2	1	1	...	16	
10. g II(B)	2.719	2.732	10. g III(B) ...	M.	...	1	...	1	3	5	4	1	...	1	16	33
				F.	...	...	1	4	7	1	2	1	1	...	17	
10. g II(C)	2.748	2.755	10. g III(C) ...	M.	...	1	3	1	4	11	5	1	2	...	28	51
				F.	...	1	...	2	3	9	5	2	...	1	23	
10. g II(D)	2.644	2.791	10. g III(D) ...	M.	...	...	1	2	5	3	1	1	2	1	16	40
				F.	...	1	4	2	7	4	1	1	2	2	24	
														Total		486
1. g III(AA)	2.779	2.651	1. g IV(AA) ..	M.	...	...	1	4	5	3	1	1	2	1	19	40
				F.	...	...	2	1	4	6	5	1	2	...	21	
1. g III(BA)	2.741	2.618	1. g IV(BA) ..	M.	...	1	...	2	3	9	4	1	1	...	21	52
				F.	...	...	1	2	7	11	5	3	1	1	31	
1. g III(CA)	2.771	2.754	1. g IV(CA) ..	M.	...	...	1	7	11	4	2	1	...	...	26	48
				F.	...	...	1	5	10	5	1	...	...	...	22	
8. g III(AA)	2.786	2.870	8. g IV(AA) ..	M.	...	2	1	4	1	1	...	1	...	...	10	25
				F.	...	...	1	1	2	6	3	1	1	...	15	
8. g III(BA)	2.593	2.911	8. g IV(BA) ..	M.	...	...	1	3	5	2	1	...	1	2	15	32
				F.	...	1	1	1	7	3	1	2	1	...	17	
10. g III(AA)	2.701	2.891	10. g IV(AA) ..	M.	...	...	1	2	5	8	4	1	...	...	21	51
				F.	...	...	...	4	7	10	5	3	1	...	30	
10. g III(BA)	2.658	2.640	10. g IV(BA) ..	M.	...	1	1	2	9	4	2	...	1	...	20	35
				F.	...	...	1	3	4	2	3	1	1	...	15	
10. g III(CA)	2.631	2.843	10. g IV(CA) ..	M.	...	...	...	1	2	4	1	1	...	...	9	23
				F.	...	1	2	1	6	...	2	1	1	...	14	
10. g III(DA)	2.712	2.935	10. g IV(DA) ..	M.	...	...	1	2	3	5	3	1	1	...	16	30
				F.	...	...	1	2	2	4	3	2	...	...	14	
														Total		336

early June of 1908. Identical methods were followed in both of the 1908 generations, with no change in the result, as far as the range in the index was concerned.

It was most surprising to find that in the different lines all carried under controlled conditions, with random matings, that the range in the index was not

in any way changed or even deformed by the removal from the organisms of the supposed forces productive of the range in the character. These results I have shown in concentrated form in tables 30 and 31.

TABLE 31.

No. of mating.	Parents, index.		Progeny.														
			Fraternity No.	Sex.	Range of index.											Totals.	
	M.	F.			2.500–2.549	2.550–2.599	2.600–2.649	2.650–2.699	2.700–2.749	2.750–2.799	2.800–2.849	2.850–2.899	2.900–2.949	2.950–2.999	3.000–3.049		
M. 2. g II	2.761 2.894 2.668	2.760 2.692 2.681	M. 2. g III ...	M. F.	1 1	4 1	11 5	16 8	31 11	11 27	9 16	5 9	3 5	1 1	... 1	92 85	177
M. 14. g II	2.708 2.692 2.923	2.750 2.774 2.661	M. 14. g III...	M. F.	... ...	1 1	3 2	9 8	22 25	12 11	4 6	2 3	1 1	... 1	... ...	54 58	112
M. 16. g II	2.778 2.810 2.746	2.764 2.689 2.891	M. 16. g III ..	M. F.	... 1	3 2	4 5	8 7	21 17	9 11	5 7	6 4	1 1	... ...	... ...	57 55	112
M. 2. g III	2.761 2.863 2.641	2.811 2.761 2.654	M. 2. g IV	M. F.	... ...	2 1	4 3	7 8	14 16	8 11	3 4	1 1	1 ...	... ...	... ...	40 44	84
M. 14. g III...	2.776 2.781 2.894	2.846 2.651 2.768	M. 14. g IV...	M. F.	... ...	1 ...	1 3	4 7	11 17	4 9	1 4	2 2	1 ...	... ...	... ...	26 42	67
M. 14. g III...	2.777 2.731 2.618	2.581 2.774 2.841	M. 16. g IV...	M. F.	... ...	... ...	2 1	4 5	10 8	6 12	5 4	1 3	2 4	... 1	... ...	29 38	67
																Total.....	619

All of the stocks went into hibernation in June and remained quiet until October of the same year. I then decided that the series was not worth the space and attention that it required, and therefore drew lots for three to be continued for two more generations in the autumn of 1908. The choice fell to pairs 8 g IV (AA), 10 g IV (BA), and mass culture 2 g IV, and these were continued for two generations more, completing the series early in January 1909. The result was in no wise changed, as is shown in table 32.

In the literature, ranges in characters of the kind used are commonly attributed to the action of incident environmental conditions of temperature, moisture and the like, and to conditions of nutrition, all of which were controlled in the series, and were uniform and optimum, and should have given uniformity and decreased ranges in the attribute under consideration if the differences were due to environment as is commonly asserted. The entire series in this direction was killed off at this point, nothing of any value being expected from its continuation.

I do not know of any other tests of this question that have been made with the same refinement, or even any tests of any sort. If I am not entirely mistaken, the instances of range in fluctuations of characters have been of reasoning from effect to cause, and instances where observed ranges were not otherwise accounted for have been attributed to this "environmental action," which

has become a dumping-ground for differences of this general class. My first proposition that "environment" was responsible for the range seems to have been answered negatively in a certain and emphatic manner.

Two possibilities remain: either the range is the product of "impurities" in the substances or due to minute differences in the constitution of the material which the methods used did not entirely isolate. I sought to test the question of the possible existence of minute "germinal variations" which might be small differences in the composition of the substance itself, through mating individuals of identical index, to determine if it were possible to obtain within the group minutely characterized races.

TABLE 32.

No. of mating.	Parents, index.		Progeny.													
					Range of index.											
	M.	F.	Fraternity No.	Sex.	2.500–2.549	2.550–2.599	2.600–2.649	2.650–2.699	2.700–2.749	2.750–2.799	2.800–2.849	2.850–2.899	2.900–2.949	2.950–2.999	3.000–3.049	Totals.
10. g VI(BA)A	2.554	2.557	10. g VII(BAA)	M.	3	11	9	4	2	1	...	...	...	...	...	30 } 59
				F	6	12	7	3	1	...	...	...	...	...	...	29 }
10. g VI(BA)B	2.679	2.681	10 g VII(BAB)	M.	...	1	3	5	9	14	8	4	1	...	...	46 } 98
				F.	...	...	2	7	11	17	9	3	2	1	...	52 }
10. g VI(BA)C	2.753	2.758	10. g VII(BAC)	M.	...	...	...	4	10	18	14	4	1	...	...	51 } 106
				F.	...	...	1	3	12	21	11	5	2	...	...	55 }
10. g VI(BA)D	2.991	2.986	10. g VII(BAD)	M.	...	...	...	...	1	3	4	9	17	5	1	40 } 83
				F.	...	...	...	...	1	2	5	10	15	7	2	43 }
10. g VI(BA)E	2.904	2.911	10. g VII(BAE)	M.	...	...	1	1	4	7	11	6	3	1	...	34 } 71
				F.	...	1	2	5	8	10	5	4	1	1	...	37 }
10. g VI(BA)F	2.893	2.891	10. g VII(BAF)	M.	...	2	1	2	3	7	14	5	1	2	...	37 } 59
				F.	...	1	2	1	5	6	4	1	1	1	...	22 }
10. g VII(BAA)A	2.556	2.553	10. g VIII BAAA ...	M.	4	17	11	2	...	...	...	...	...	...	...	34 } 77
				F.	7	19	13	3	1	...	...	...	...	...	...	43 }
10. g VII(BAB)A	2.677	2.678	10. g VIII(BABA) ...	M.	...	1	1	3	4	11	5	4	1	2	...	32 } 67
				F.	...	1	1	4	10	9	5	3	1	1	...	35 }
10. g VII(BAC)A	2.753	2.756	10. g VIII(BACA) ...	M.	...	...	1	1	10	15	11	2	...	...	...	40 } 85
				F.	...	...	1	3	12	19	9	1	...	...	...	45 }
10. g VII(BAD)A	2.989	2.993	10. g VIII(BADA) ...	M.	...	...	...	...	...	1	3	12	21	11	1	49 } 100
				F.	...	...	...	...	...	...	2	14	20	13	2	51 }
10. g VII(BAE)A	2.901	2.912	10. g VIII(BAEA) ...	M.	...	...	1	2	1	3	5	2	2	1	...	17 } 42
				F.	...	1	2	1	2	4	7	5	1	2	...	25 }
10. g VII(BAF)A	2.895	2.890	10. g VIII(BAFA) ...	M.	...	1	1	4	6	11	5	1	2	1	...	32 } 57
				F.	...	1	...	1	3	5	8	3	2	2	...	25 }
															Total	 934

In October 1907, I mated from stock 6, line 10 g VI (BA) (table 32), 6 pairs, when both parents had the same index, but reared under the conditions of the general culture quarters. The progeny obtained from these pairs matured at the end of November and showed some indication of the influence of the method that had been practiced. Early in December I again mated from the same lines pairs having the same indices as in the first instance, and obtained a second generation early in January 1908, in which there were unmistakable indications of the action of the operations in some of the lines, but by no means in all, a condition that might mean one of several possibilities. All hibernated from the middle of January to the first of March 1908, when they were forced from hibernation and mated. In table 33 I have shown the conditions of the series in the two generations of 1907.

Three lines shown in table 33 showed distinct tendencies to become restricted in their ranges, and these three, 10 g VIII (BAAA), 10 g VIII (BACA), and 10 g VIII (BADA), and were preserved for further analysis to see if continued operations of the same kind would further restrict the range in the fluctuations of the index. The remainder of the lines were killed off or used for other work, having given no indications of curtailment of the range as a result of the operations. The three lines reproduced between the middle of March and the middle of June, gave two generations in which the same standard of parental indices were maintained in both generations, with the result that at the end of the tenth generation in June 1908, three clearly defined groups had resulted, and which

TABLE 33.

No. of mating.	Parents, index.		Progeny.												
					Range of index.										
	M.	F.	Fraternity No.	Sex.	2.550–2.599	2.600–2.649	2.650–2.699	2.700–2.749	2.750–2.799	2.800–2.849	2.850–2.899	2.900–2.949	2.950–2.999	Totals.	
8. g IV(AA)	2.625	2.851	8. g V(AA)	M.	1	4	5	8	7	4	1	1	...	31	64
				F.	1	1	6	11	8	3	2	1	...	33	
10. g IV(BA)	2.770	2.741	10. g V(BA)	M.	...	1	4	7	12	8	5	2	...	37	72
				F.	1	3	6	10	7	5	1	1	1	35	
8. g V(AA)	2.753	2.761	10. g VI(BA)	M.	2	4	6	11	7	5	3	1	...	39	75
				F.	1	3	7	14	5	4	2	...	...	36	
10. g V(BA)	2.701	2.626	10. g VI(BA)	M.	2	8	5	8	16	7	6	4	2	53	103
				F.	2	5	7	19	8	3	2	2	2	50	
M. 2. g IV	2.650 2.761 2.883	2.741 2.789 2.675	M. 2. g V.....	M.	1	3	5	18	11	10	6	4	...	58	133
				F.	1	4	7	9	21	17	10	5	1	75	
M. 2. g V	2.681 2.891 2.714	2.741 2.814 2.752	M. 3. g VI.....	M.	1	3	8	11	7	5	1	1	...	37	68
				F.	1	4	6	14	3	1	1	2	...	31	
														Total.....	515

could no doubt have been maintained in the same condition and range of index as long as required. The differences between the three strains were so small that it was not possible to certainly distinguish them in the cultures by inspection, and only measurement could reveal the true state of affairs.

The condition in these three strains continued through the four generations that they were carried, showing that there were minor differences in the original "biotypic group" which could be isolated by more minute methods of analysis and perpetuated by common methods of isolation, proves that the original "fluctuations" are not the simple set of "environmental" disturbances they are sometimes asserted to be.

In the autumn of 1908, after hibernation since June, the three cultures were again put to breeding, and two more generations of precisely the same character were produced, indicating further the constant nature of the small differences that were being dealt with. In the last three generations it was found that there was a tendency for the groups with the higher and lower indices to transgress the bounds of the variability of the original "biotype" stock, espe-

cially in the direction of a higher index, which was away from the condition in the species as a whole. It would have been of interest to have followed out this suggested tendency in the upper group, but space in my laboratory was at a premium, so that the entire culture at the beginning of 1909 became the victim of a struggle for space. In any event the culture had shown clearly the main point at issue, namely, the presence in the population of minute differences, fixed in the germinal constitution of the stock and capable of being isolated, and held as distinguishable groups when the proper refinement in method was employed, showing clearly that the "fluctuations" are not all uninheritable in these "biotypic" lines.

It must be remembered that these lines were not "genotypes," and as "biotypic" lines are not fully comparable with the "genotypical lines" with which Johannsen, Shull, East, and others have worked, and concerning which so many of the recent qualifications have dealt. However, as most narrowly delimited lines of biotypical breeding, they are good examples and do show, in these lines, that the problem of the "fluctuations" is by no means as simple as it might seem from some rather emphatic statements as to the limitation and nature thereof.

The issue might be raised that the original group was not a true biotype at all, but a complex assemblage, and this may be true; but where is the limit to be drawn? With the ordinary methods in use the first group satisfied the requirements fully, and remained as a constant assemblage with the customary range in the index after once it was established without further attention or selection on the part of the breeder. If I had gone further no one would for a moment have even thought of the group being anything but a simple one. Nor do I know how complex it was in any instance, nor how many of these minor strains I could have isolated from the mass of a single population, with time and refinement of method. In all of the pure-line work the same seems to be true, that with refinement of method the lines may well become finer and finer, less and less remotely separated, and lines only in the sense that our methods of analysis and pure breeding hold them as such, and in a most unnatural condition.

The third possibility remains, that some of the "fluctuations" may have been due to loosely combined "impurities" in the constitution of the lines, which would in this instance be in the nature of substances present as "factors," which, not being in the proper place and relation in the system, or lacking the proper complement, gave no direct resultant of their own in the reactions of the other "factors" of the mass, but by their presence served to disturb the normal workings of the system and thereby produce distortion of the action and deformation of the resultant "character." It is well known that in the non-organized bodies, this type of deformation is common; indeed is the most common one in nature, and there is no *a priori* reason why the same may not well be true in organized matter, although not so easy to test or to certainly determine.

In the culture 10 g VII (BAF), and again in the next generation of the same line, as shown in table 32, a wide range in the index is shown, and the same method that produced the restriction of the range in the other cultures had failed in this one, so I tested it for two possible causes of the condition found.

First, four pairs were put into controlled conditions and carried through four generations with the results shown in table 33, and the graphic representation

in figure 33A. No decided influence of the uniform conditions in restricting the range of the index was found, the range at the end being about the same as at the start.

Second, there was the possibility of "impurities" in the constitution of the strain. I went over the living stock carefully and over such of the preserved

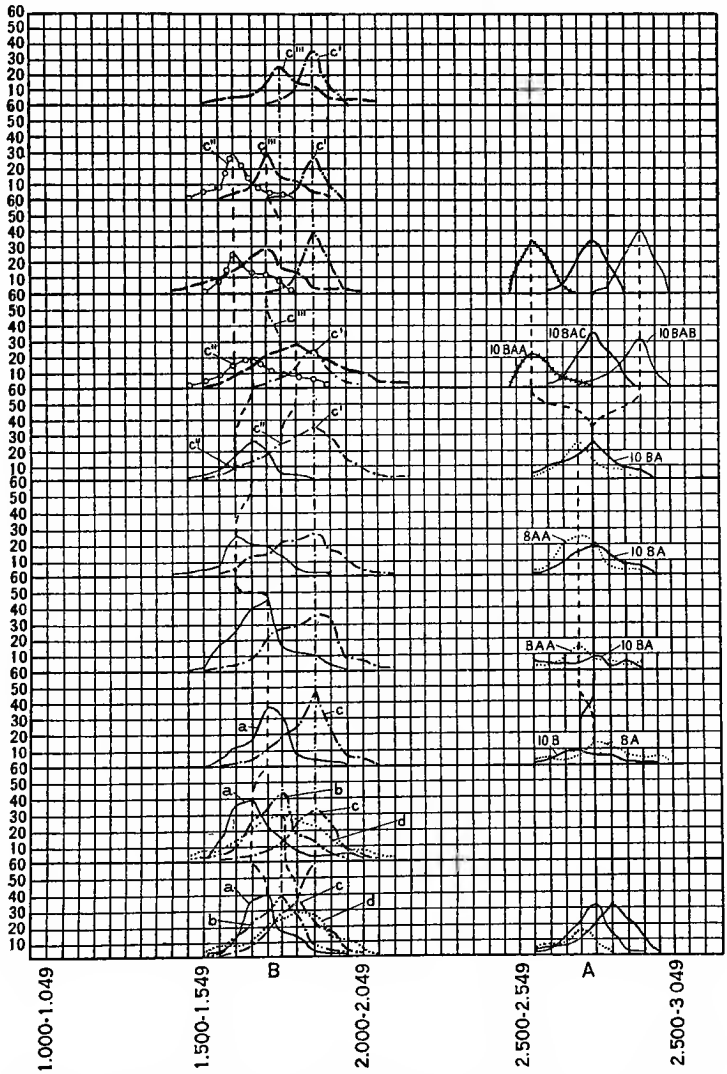


FIG. 33.—Diagrammatic representation of results obtained in the attempt to breed pure lines as regards form-index in *L. multitæniata*. (See text.)

material as I had, and found in this "line" a suggestion upon which I went to work. In all of the biotypical lines of this *multilineata* series the spots *a* and *a'* are parallel with the median axis of the body and are also moved close thereto. I found in this strain that these two spots were in many of the specimens somewhat divergent at the anterior end and were, on the whole, more widely separated

than in the other lines. I knew from other portions of this investigation that the position and relation of these spots is directly the product of a complex set of body form-factors and that this complex set was capable of dissociation, and that in some instances the factors went far astray as loosely attached members in other portions of the system. There was no possible method of even guessing at what the specific cause of the disturbance might be in this instance, if it were that at all, and there were so many possibilities that any attempt to test out its nature within the species *L. multitæniata* was hopeless or at least not hopeful. I tried the tests with another species, *L. oblongata,* in which there is not present the factors productive of the wide separation and divergence of the spots *a* and *a'*. In this form they are always parallel and closely set to the median line. I had some pure-line cultures of this species at that time and crossed the suspected material with the *L. oblongata* lines, and the F_1 results showed at once that the suspicion was well founded, as the four fraternities reared showed strong indications of the *multilineata* type of pattern; and in the F_2 results some forms came out in the progeny that were in pronotal pattern *multitæniata* in every respect, with the widely divergent *a* and *a'*, as well as in other characters. The result was clearly an indication that the "biotype strain" had something in it that gave, in the new opportunity which cross-breeding gave it, the capacity to form new associations and initiate diversity of characters, while in the first position its location in the system, or lack of proper complements allowed only manifestation by the lack of stability found in the series of narrowly restricted strains.

Thus far I had found only a possible source of contamination, but did not know what the contaminator was, excepting that it visibly deranged the two spots mentioned, a rather trivial indicator. It was hopeless to try to purify the strain through the use of neo-Mendelian methods, as the characters were too fine for certain recognition, and the fact of crossing would only introduce additional complications. The only resort left was the effort to purify the race through the elimination in the breeding members of any indication of the contamination as revealed in the spots *a* and *a'*. This I began early in 1909, paying especial attention to the character of the spot system, with the result that at the end of 1909, having put the stock through four generations, that the range in the index had fallen to less than half of its former range, even though the indices of the mated pairs had not been so rigorously coincident as in other matings. The figures from these generations are shown in the table 34.

After hibernation from the end of December 1909 to the first of April 1910, the stock was put to breeding again, this time without any attention to either pattern or index, mated at random for the first mating. The progeny showed no change; and again mating as a group culture, the last generation reared showed only the same narrow range in the index. This rather rough treatment of the line gave some indication of its purity and fixity, as well as the freedom from the formerly present factor or factors that had been productive of the wide range in the index in the earlier generations, and there is no doubt that the process of elimination carried on as I had begun it would have resulted in the formation of a race that was free from this one impurity and would have been narrow-ranging in the index, as were some of the others.

Not all of the lines gave results as easily as this one, or even were capable of solution at all. Thus, the line shown in 10 g VII (BAB), table 33, was of the same wide range in the index, but the same source of impurity was not present,

or at least was not to be detected by the same methods. It was tested with controlled conditions to no purpose, and no clue was found to the range in the index present, so that selection through 1909 and 1910 failed to change the array in any respect. This may be interpreted in different ways, but the range was surely not environmental, else it would have shown at least an indication to respond to regulated conditions, which it did not at any time; or if smaller germinal differences were present it must surely have been possible to have done at some time the same here as in other lines, but more probable seems the hypothesis that there was some "impurity" present, which my methods of detection did not reach, and which by its presence in the system continued in spite of rigorous methods to produce wide ranges in the index of this biotypic line.

The results in this series were a decided surprise to me, and indicate clearly the difficulties of the problem and the immense complexity thereof. As far as the cause of the "fluctuations" was concerned, the outcome was not expected or anticipated on the basis of the assertions made regarding these differences in organisms, and the quite general independence of the index from environmental influences is striking, as is the possibility of finer divisions and the presence of impurities. It would be of interest to completely analyze the nature of the entire fraternity resulting from any pair of parents, and I have often thought that it would be worth while to attempt it, but I have never been able to obtain the space or time free from other work to allow of the attempt being made.

At the same time that the experiments with the *multilineata* biotype series were in progress, another part of the same species gave interesting confirmation of the same principles, but, as might be expected, with diversity in the details. In the course of the preliminary testing of this species I had separated out a form "biotype" in which the index was low, on the average about 1.750, with a range upwards to about 2.000 and on the lower side to about 1.550. The same elimination of color and pattern differences were practiced, so that at the end of 1906 I had a well-defined set of four races of this material, which were utilized in some tests, that are companion to those already presented.

The original stocks were uniform in aspect and in behavior in crosses and in every respect appeared as homogeneous racial line cultures, and all showed essentially the same "amount" of "fluctuations" in each fraternity, as shown in figure 33B, in the pairs *a, b, c,* and *d;* and in the following generation progeny of matings from these same fraternities showed the expected range in the "fluctuations." These were random matings within the population, reared under the conditions of the general breeding quarters, without any control of the conditions. I then reared them through four generations under the same conditions as in the *multilineata* series, with the same general result in the two lines carried from pairs *a* and *c*. This difference was found in that in the line *c* the modal condition remained at the same point on the scale, while the mode of the *a* series was constantly shifting its position. Neither of the lines showed the slightest indication of restriction in its range as a result of the controlled and more uniform conditions of development, so that the environmental influences were apparently not operative in this set of "fluctuations" more than in the first one.

The next step in the testing was the attempt to breed minor races from the lines, as was done in the first series. With the line *c,* three sets of matings were made from three points in the array of the fraternity, as shown in figure 33B.

Two of these gave at once the results that had already been obtained in the other series, giving in the sublines c' and c'' fraternities of decreasing range in the index and increasing isolation from one another. The third mating, c''', did not respond to this treatment, and throughout the test retained its range in the fluctuations and showed considerable shifting of the mode during the course of the four generations. The c subseries shows, at any rate, that in the population there were present minor conditions that could become fixed as minor races by the use of appropriate and delicate methods of analysis and isolation. There were clearly present in the same fraternity those that did not respond to the same treatment, and which retained the range in the index and showed a shifting mode. It is clear that the condition in the original c line was not a simple one, and this may be entirely responsible for the failure to respond by decreased fluctuations to the conditions of the medium when held uniform.

From the a line four matings were made, only one of which was found to respond to the conditions of the effort and become restricted in the range of the fluctuations as in other series, while the other lines preserved their original range of index and the shifting of the mode, so that the result, which is not represented in the figure, indicated only the complexity of the assemblage, the absence of environmental influences, the presence of minute fixable differences, and suggested the existence in the strain of agencies invisible in manifestation as to characters in the usual sense, but productive of unstable conditions, admitting of such diversity in the range of the indices under investigation.

The entire series is suggestive of the conditions that may be expected in a large number of characters, and the findings are more in line with the conditions found in the simplest color-characters than might at first have been expected. From time to time other series of determinations have been made in the effort to analyze these problems of " variation," and thus far with identical results. In the juvenile stages similar dimensions, especially the head-breadth, have been the basis of some similar examinations, with entirely similar results. It is not necessary to add to the already rather long mass of data presented, in that nothing new in principle is gained and added cases are not of interest at this place. The analysis of the meaning of heterogeneity in these simplest characters has shown interesting developments in this material, but further discussion and analysis of the situation is best deferred until after some data of heterogeneity in systems of pattern and structure have been described and analyzed; then it will be profitable to return to an analysis of the entire problem of heterogeneity in the individual and the population.

CHAPTER VIII.

ANALYSIS OF HETEROGENEITY IN COMPLEX CHARACTERS.

COMPOSITION AND MODIFICATION OF THE PRONOTAL PATTERN.

The pronotal color-pattern of this group, the genus *leptinotarsa,* affords an opportunity for the investigation of heterogeneity in a highly "variable" organic "character." The species as a rule are narrowly limited in distribution, and some show minor habitudinal divisions. Within their habitats they live in restricted colonies, as described in Chapter II, and are, therefore, localized in differing habitats between which there is little interchange of populations. These conditions produce isolated populations in which it is possible to test some of the relations between the "variation" phenomena and the conditions of existence. The pronotal color-pattern is chosen as a subject of observation because of its composition of exactly localized elements and the ease with which differences may be estimated, seriated, and recorded. It is a complex of simplest characters, some of whose departures have already been analyzed. The pattern of this part may be considered as a system largely independent of any of the other parts.

IN LEPTINOTARSA MULTITÆNIATA.

In species like *L. multitæniata,* examination of a collection of a thousand or more individuals, taken at random over its geographical range, shows a wide range of pronotal pattern, as shown in figure 34. Conditions from a totally

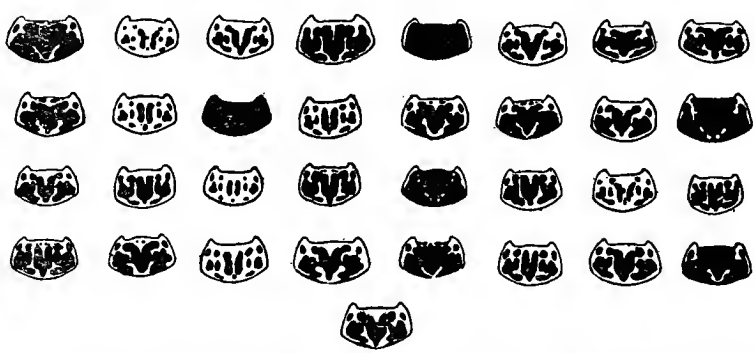


FIG. 34.—Showing great diversity in color-pattern in *L. multitæniata* throughout its range. The first appearance of these patterns on inspection would seem to warrant the view that their aspects were entirely without order or uniformity.

black surface to one with only the simple spots present are found, and between these are all sorts of patterns, of differing amounts of pigment, and differing combinations between the elements. In a given generation in some locations in a large sample it is possible to find all or nearly all of the variations shown, while in other locations and generations only a few are present. The treatment of a population showing a character of this diversity depends largely upon the point of view and the philosophical background applied to the problem.

The simplest and easiest method is to assume that this condition represents wide fluctuation in the quantity of pigment and that it spreads in all directions, producing the fusions and different patterns found. Upon this basis the essential phenomenon is the amount of pigment and the variation is one of quantity. In my 1906 paper, page 99, the place variation of *L. multitæniata* at Guadalupe, Federal District, Mexico, is given for six generations, *i. e.*, 1903, 1904, and 1905, from the biometric standpoint, and is expressed in terms of the area of the pronotum covered by the dark pigment, no pigment being 0 per cent and entirely black 100 per cent. When seriated the successive generations show only that the amount of surface colored black varies from generation to generation; that the polygon of distribution of these quantities shifts plus and minus with different means and modes and unlike curves. These determinations I might have plotted in curves of distribution, but from them no further information can be obtained, and no amount of mathematical treatment can extract a single additional fact of interest. All the essential facts of the different patterns presented in these years, the way in which these patterns reacted in the reproduction of the population, and the real relations of the elements composing the pattern are lost in the statistical methods employed.

In the years 1903 to 1910 observations upon the heterogeneity of several tropical species were made, together with the attempt to analyze the findings. In the northern form, *L. decemlineata,* similar analyses of the variation at Chicago from 1902 to 1907 were made. It was the experience with *L. decemlineata* from Massachusetts and Chicago that gave the original clue to undertake a more extensive analysis with the more favorable materials of the Mexican tropics.

L. multitæniata, as shown in Chapter II, is variable in composition, is limited in distribution to the Mexican Plateau, where its actual habitat is localized, so that little interchange between different colonies takes place. From 1903 to the close of 1910 continuous observations were made upon this species in several locations. By continuous observations I mean study and actual contact with this species in nature at stated periods in each of its two annual generations throughout the entire period. This necessitated either actual residence in the country, as in 1903, 1905, and 1906, or two or more visits to Mexico per year for purposes of observation. A series of from 10 to 16 consecutive generations were thus tested and analyzed in this investigation.

LOCATIONS FOR OBSERVATION.

In 1903 a series of locations were selected after a preliminary series of observations had been made, and actual work was begun in 1904. These locations were chosen along the southern edge of the Mexican Plateau, and presented a diversity of conditions. At these locations two sets of problems were studied: (1) the analysis of heterogeneity in the individual in different locations; (2) the analysis of heterogeneity in the population through a period of years. The two are interdependent, and especially is the second dependent upon the first. The locations selected were as follows:

A. Valley of Mexico: Chapultepec colony, Tlalnepantla colony, Texcoco colony.
B. Rio Atoyac Valley: Puebla.
C. Slopes of El Volcan de Citlaltepetl: San Andres Chalcicomula.

These locations are shown in plate 19, and while close together and easy of access by transportation, are diverse in their conditions of life, so that as far as possible any influence of localized conditions or other environmental factors could be detected and made the basis of experimental analyses. The three general regions were the Valley of Mexico, Rio Atoyac Valley, and the western slopes of Volcano of Arizaba.

The Chapultepec colony—A location 1 kilometer southwest of the Palate of Chapultepec (near Mexico City), in the open plain just above an old shore-line, of Lake Texcoco, was chosen in 1903, and regular observations were made beginning with the first generation of 1904 and continued without interruption until the middle of 1910. Thirteen generations were thus observed. This location is an open savannah, sloping from the hills to the west of the valley towards Lake Texcoco. It is well watered by rain and less influenced by drainage operations than the location about the Sierra Guadalupe. Further, the observations of the meteorological conditions obtained at Tacubaya were fairly applicable to the location.

The Texcoco colony.—Another location was chosen in 1903 on the east shore of Lake Texcoco near the town of Texcoco, Federal District, Mexico. Two reasons determined this location; first, its being an old lake-bed and still at about the lake level, so that it differed strikingly in soil-water supply from the Chapultepec Station, and second, the fact that fairly systematic meteorological observations were made in the town. These latter, although not exactly what I would like to have had, were still a fair measure of the difference between the two localities, one to the west and the other to the east of Lake Texcoco. Observations were begun in 1903 and continued until the close of 1909 or 13 generations.

The Tlalnepantla colony—In the north-central portion of the basin near Tlalnepantla, Federal District, Mexico, where meteorological observations were also to be had, a location was chosen near the Rio Tlalnepantla, along the border of some cultivated fields. Observations were begun here in 1903 and continued until 1907, 10 generations being under observation. In addition to these locations records were also continued near the foot of the Sierra Guadalupe until the end of 1907, or for 8 generations. Other records were also made for locations near San Angel, Santa Fé, Tlalpam, and Huehuetoca, also in the valley of Mexico; at all, results the same in principle but not in detail were obtained, and these, together with the fact that meteorological observations fairly applicable to Chapultepec, Texcoco, and Tlalnepantla were available, led to the concentration of effort upon the colonies at these points in the valley of Mexico.

RIO ATOYAC VALLEY.

Only one location near the city of Puebla, State of Puebla, Mexico, was established. A number of locations were examined, but the absence of climatic records of any value and the difficulty of having them made decided the location of the Puebla colony. On the open plain about 4 kilometers north of the city, in uncultivated land used for pasture, a thriving colony was found, and to the climatic conditions of this location the meteorological records of two stations in the city of Puebla were fairly applicable. Observations were begun in 1903 and continued until 1908, or 12 generations in all.

SLOPES OF EL VOLCAN DE CITLALTEPETL.

On the cold, dry, grassy slopes on the western side of this huge volcanic cone a small colony was found near the station of San Andres Chalcicomula, State of Puebla, Mexico. This location, although without meteorological records, is of interest because of the cold, dry, tundra-like area in which it was located, the short growing-season, and the generally rigorous conditions of life. Observations were begun in 1904 and continued until 1909, 12 generations being observed.

Observations were made at a number of other points: At Ometusco, Federal District, Mexico, 1903-1905; Apizaco, Tlaxcala, Mexico, 1903-1907; Esperanza, State of Puebla, Mexico, 1904-1907; all on the open high plateau; at San Martin, State of Puebla, Mexico, 1904-1906; and Tlaxcala, State of Tlaxcala, Mexico, 1904-1906. The purpose of these was to discover if there were different processes at work in locations other than the five chief points of observation. None was found, and it was not thought wise to expend time and energy upon more than the five locations. At each of these colonies exactly the same method of observation was applied, and as far as possible observations were made at all of the colonies, especially the five important ones, at as near the same time as possible. This was made fairly easy by the rather good railroads operating in that portion of the country, and without these the project would have been impracticable, if not impossible.

METHODS OF OBSERVATIONS.

It has been the universal custom in studies of this character to make collections in the locations chosen and preserve them for study later. In this way a considerable proportion of the population of the colony is removed and an unknown population of undetermined character left to continue the species at that point. In organisms as localized as these are, and often inhibited from extensive dissemination by unfriendly environs, it is conceivable and highly probable that the removal of large numbers from a restricted area would introduce complications not easily determined or compensated for. It was decided at the beginning to eliminate any error of this nature by not removing any of the population, or at least in any numbers, and only those needed for experimental testing. This plan has been rigorously followed and made necessary the determination of the condition of the population in the field. This was easily accomplished. Two, three, or more natives were instructed to collect all of the kind of beetles they could find in a given area, and those alive and uninjured were recorded by me, and the sex and type of variations presented by the part were examined. These after examination and recording were kept caged until the census of the colony was complete, and all were then returned to the colony to breed and do whatever other things they might. I am therefore certain that the conditions described are those resulting from causes proper and natural to the location, and that no error has entered into the observations by elimination on my part from making collections.

At Irolo, State of Hidalgo, Mexico, I made a test in 1904, 1905, and 1906, removing a large part of the population from the colony, and by the end of 1906 it had become very weak in numbers and its character otherwise much altered. It seemed on the whole best not to remove from any colony under observation many individuals in any given generation.

At the start two methods of recording for this part were used: (1) an exact record of the type of pattern found and the extent and direction of the unions between the component elements; (2) a record of the proportion of surface pigmented to that unpigmented, a purely quantitative statement of the "variation" in the amount of pigment present. This latter determination, estimated on the scale of 0 to 20, was abandoned at the close of 1906, the biometric results being utterly meaningless and of no significance whatever.

Each year, during the term of observation, the colony was visited at or near the time when the first and second summer generations were at their maximum in number. The life-history, Chapter II, shows two annual generations of this form; the second, passing the dry season in hibernation and emerging in late May or early June, become the parents of the first summer generation, which in turn breed at once, giving the second summer or hibernating generation. In nature it is not always possible to be entirely sure that the population examined is entirely of a given generation, because of the fact that a few of the overwintering generation may live on and mix, and even breed with the first summer generation. These can commonly be separated by the distinctly duller color and generally aged appearance. Some, however, may have escaped detection, and have been recorded as first-summer generation. So, too, the first generation may mix with the second. As a rule these mixings can be detected easily in the living specimens, but a few possibly have passed into the wrong records.

Having established the general plan of investigation, the next step was to determine what variations were present in the pronotum of this species over its entire range. Accordingly, in 1903 a collection of over 8,000 specimens of this species was made from all portions of its geographical range and these carefully analyzed, and from this collection it was found that several distinct pattern-plans were present, and upon these pattern-plans much, if not all, of the variations found were based. In figure 34 is shown an array of these variations. In *L. decemlineata* I had previously found that the variations could be arranged around several central plans, which in turn could be arranged in a large scheme covering the entire range of variations of the species. In *L. multitæniata* 12 such pattern-plans exist, as shown in figure 35, and these are in turn capable of being arranged in a scheme of "variation" of the whole species as regards the pronotal color-pattern, as shown in figure 36. It was further found that, using figure 36 as a basis of plotting, a far more accurate expression of the character of the population as regards this feature was possible than by any other means. It expressed well the direction, kind, and extent of the variations presented by this part. In figure 36 are given all the variations found in the species, and only rarely and in large populations are all of these ever present at one time. It was found that the variations of this species could be accurately separated by this method of plotting, precisely as had been previously used in *L. decemlineata.* Blanks of this form (fig. 36) were prepared and taken into the field, each individual being entered on the proper part of the blank and also on the second record, indicating whether in the given pattern, the amount of pigment was increased or decreased below the average for that pattern. In this manner there was obtained by direct seriation a statement of the number of individuals showing a given pattern and the variations of that pattern towards others, and also in the quantity of pigment present.

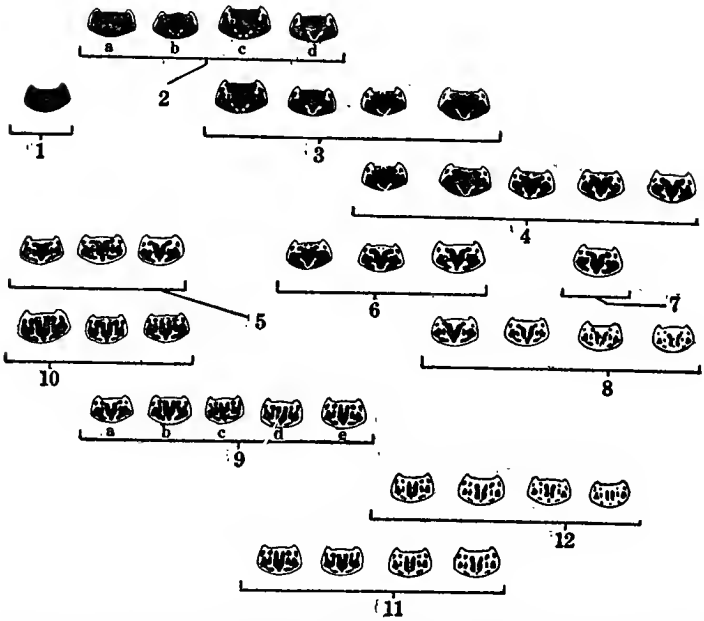


FIG. 35.—Showing the 12 biotypic groups into which it has been possible to separate the pronotum pattern of *L. multitæniata*. These groups are based upon the fact that each has been isolated from the general population and reared in pure homozygous acting pure lines through several generations in each, and represent with present methods all the stable groups of the pattern as a whole that I have been able to isolate.

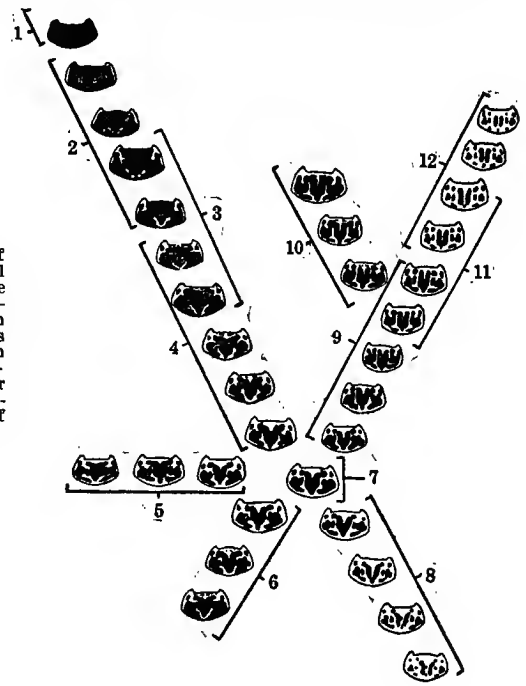


FIG. 36.—Showing arrangement of the 12 biotypic groups into a general scheme for the population as a whole and different directions of combination and variation within the pattern as presented by the population. This schematic base showing the position of the 12 biotypes within the population has been used as the basis for recording the heterogeneous conditions found within the population of this species in nature.

From time to time materials were taken from each of the colonies to Chicago, and there tested experimentally to determine the heritability of the pattern and the gametic constitution of individuals showing it. Pure races of different patterns were obtained, and these, after testing and analysis, gave a good basis for the interpretation of conditions found in nature.

COMPOSITION OF THE COMPLEX PATTERN.

An abundance of instances of complex patterns or parts have been recorded and arranged in a system, a common practice in studies where phylogeny is the basis of arrangement and perspective is limited to some system of phylogeny and hypothesis of evolution. It is here attempted to unravel the intricacies of the complex pattern of the pronotum of these beetles in the effort to discover the laws governing the obvious heterogeneity. It has been shown (fig. 34) that in *L. multitæniata* there are many patterns giving seeming diversity, and that these can be arranged in 12 series (fig. 35); and further, that these 12 biotype groups of true-breeding types can be further combined into a pattern or scheme expressing the whole range of diversity in the entire species (fig. 36), and upon such a scheme could be graphically represented the variations of the species in any particular location and generation.

One conspicuous fact of this arrangement is the divergence from biotype type 7, through types 4, 3, 2, and 1 to a totally black surface, through types 9, 10, 11, and 12 towards a different extreme, and through 5, 6, and 8 towards still other divergent types. In any population there is evident a complete gradation along any of these lines towards the extreme type, and perfectly continuous series in some of them are at all times evident. The first question to be decided was, is this population a unit with a complete gradation towards some race extreme, or is the population a composite of lesser types which, assembled, produce the scheme of the whole and its apparent "continuous variations"?

I had found much earlier that the simpler population of *L. decemlineata* could be broken up into several lesser groups, depending upon the characters used and the degree of rigorous inbreeding practice. Were these groups natural or artificial? The work of Johannsen and De Vries raises this question in more urgent form. De Vries postulates "elementary species," and such divisions are found in this species, while Johannsen postulates lesser lines, biotypes, genotypes, and clones, of which biotypes only are found in sexually reproducing species. This material has afforded an opportunity to put to a test some of the recent developments in genetics.

A postulate common to the conceptions of De Vries, Johannsen, and all of the neo-Mendelian workers is belief in the inability of a minor strain, when pure, to be modified by continued selection of the extremes of its fluctuations. The problems are: (1) can races breeding true without constant selection be obtained; (2) when obtained, can these be modified by the selection of extremes?

One of the most striking characteristics of this material is that when obtained in nature it rarely breeds true with respect to a specific type of pronotal pattern, and usually from a pair of wild parents different types appear in the progeny. *A priori*, this may be due to wide "variation," or hybrid constitution of the parents respecting the types of pattern represented. Breeding will show accurately which one of the two is correct.

Figure 37 shows in schematic form the results from the progeny of a single pair, both of which were in aspect type 1 pronotum. A pair of wild beetles with black pronota from Chapultepec [1] were bred in 1905-06, and gave in F_1 the array shown in the diagram, with some fluctuations of each of the five types which are represented. Of the five types found, examples of each were mated with likes. A pair of black type 1 was mated (A), and gave in F_2 equal numbers of black A' and a type 2 form A''. Each of these when inbred gave in F_3: from the black of A' came 100 per cent black, and this continued to breed true, while from the type $2A''$ came black A'' and type 2 again $A''a$, in about equal numbers. The blacks in F_3 A'' bred true for three generations, while the type $2A''a$ split

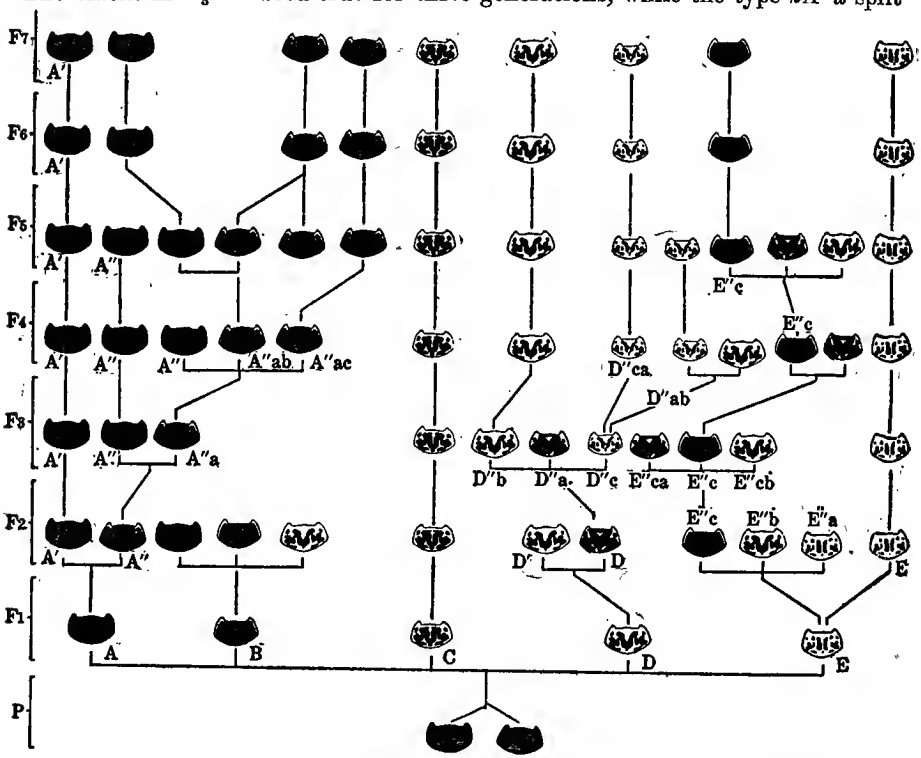


FIG. 37.—Schematic results showing complex nature of a pair of *L. multitæniata*, having a pronotum of biotype 1 and heterozygous in nature.

into blacks $A''a$ and type 2 again, some of which $A''ac$ continued to breed true, and some $A''ab$ split again. In F_2 of type 2 two pairs were mated and only one gave progeny, and in F_3 two pairs were again mated and only one gave progeny, but in F_4 six pairs out of eight gave progeny from type 2 matings and two gave the behavior shown in $A''ac$, and four that of $A''ab$. Two races, stable, without selection were obtained from A.

From B no pairs were mated, but from C in F_1 out of three such matings one bred true and gave a stable race. From D, of which I bred only one pair, two types appeared in F_2, a low extreme of biotype 4 (D') and a biotype 3 (D'').

[1] In all tests of materials from nature the larvæ were taken and reared, and the sexes isolated at emergence, so that the females were in all cases certainly virgin.

The biotype 4 (D') was not mated and one pair of biotype (D''), which gave progeny, gave in F_3 biotype 2 ($D''a$), biotype 3 ($D''b$), and an extreme of biotype 8 ($D''c$). One pair of F_3 of the type 4 ($D''a$) which gave progeny was the basis of a line breeding true and constant. The other pairs gave biotype 4 and biotype 3 (not shown). Three matings of biotype 4 (DEa) gave results like similar matings in F_3. The biotype 8 extreme ($D''c$), of which there were only two pairs that gave progeny, gave in one case all biotype 8 extreme ($D''ca$), and in the other pair ($D''ab$) two biotypes, 8 and 4, appeared, which, however, were not fully tested.

From the mating E in F_1 the results from two pairs are shown, one (E') breeding true in F_2 and subsequently; the other giving in F_2 a population of a biotype 12 ($E''a$), a biotype 4 ($E''b$), and an extreme biotype 2 ($E''b$). Of these only the extreme biotype of 2 ($E''c$) were mated and one pair gave in F_3 biotype 2 again ($E''c$), a biotype 3 ($E''ca$) and the other extreme of type 4 ($E''cb$). Only the biotype 2 ($E''c$) was mated in F_3, and this pair gave in F_4 biotype 2 ($E''c$) and biotype 3. In F_4 five pairs of biotype 2 were mated, one of which gave biotype 2 ($E''c$) and types 3 and 4, and of this F_5 population matings of the biotype 2 ($E''c$) showed one that was constant in F_6 and F_7.

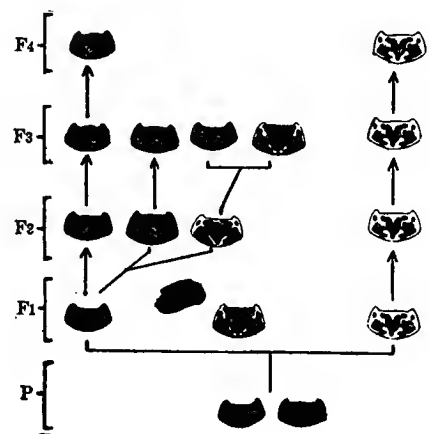


FIG. 38.—Showing results in a second pair of *L. multitæniata* from the Chapultepec colony, having a pronotum of biotype 1; although obviously heterozygous in composition they were apparently much less complex than the similar pair shown in figure 37.

It is evident that the original pair[1] were not pure black type 1, but complex hybrid combinations in which different potentialities were combined in such a series of complexes that the chance union of these two gave rise to a striking array of types, out of which several distinct races were extracted and maintained as pure-breeding strains. It is also shown that although the original stocks were exceedingly heterozygous in character, their progeny were, nevertheless, quite fixed in type and the array found was entirely due to diverse germinal constitution.

Although the pair and their progeny show extremely complicated germinal constitution, not all tested pairs were so complex. Thus in figure 38 is shown a pair obtained at the Chapultepec colony in 1908, which gave a simple behavior,

[1] Both of these were typical "*L. melanothorax* Stål" as found in all museum collections and would unless tested by breeding have passed as pure typical specimens of that "species."

showing simple gametic composition. In this pair of biotype 1 blacks from nature were mated and gave in F_1 biotype 1, biotype 2, and biotype 6. The F_1 blacks when inbred gave, out of 7 pairs, 4 that came true and 3 that gave blacks and type 2. In F_3 and F_4 the pure-breeding blacks continued to breed true and the type 2 was heterozygous, giving type 2 and blacks of biotype 1, the latter breeding true. Of the biotype 6, which appeared in F_1, 2 pairs out of 8 gave progeny, and these came true to type in F_2, F_3, and F_4. The biotype 2 in F_1 inbred gave biotype 1 and biotype 2 in F_2, but these were not mated for further testing. The simple behavior of this pair shows a relatively simple gametic composition and the capacity for extracting pure-breeding biotypic races by the mating of likes.

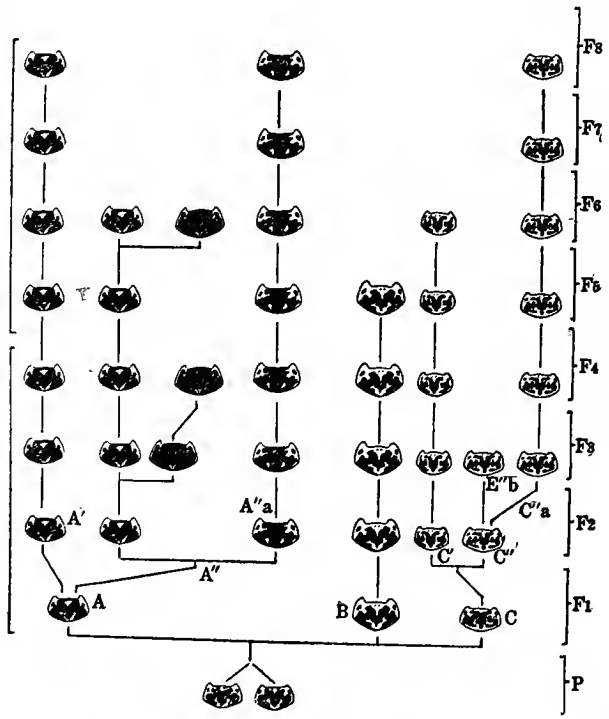


FIG. 39.—Showing results obtained from a single breeding pair of *L. multitæniata* from the Chapultepec colony of biotype 4.

Even more simple cases have been found where blacks of biotype 1 were pure homozygotes, but in nature these are not common, but may be found in almost any population of emerging stock by testing enough samples. It is certain, therefore, that as regards this biotype that it exists in nature in all degrees of gametic constitution from pure homozygous to highly complex and intricately involved heterozygous constitutions, where there are correlations with other characters, giving extreme diversity in the progeny of a single pair. These correlations, while of importance in the production of diversity in the pronotal character in the population, can not be discussed in this part of the paper.

The conditions found in the biotype 1 pattern are equally true of any of the others found in the entire population. In figure 39 are shown the results

obtained from a pair of biotype 4, also from the Chapultepec colony, as wild stock. In F_1 this pair gave biotype 3 (*A*), biotype 4 (*B*), and biotype 5 (*C*). In F_2 two pairs of biotype 3 (*A'*) were found that gave 100 per cent of this same type and continued to do so through F_4, F_5, F_6, F_7, F_8, and F_9, while 5 pairs *A"* gave biotypes 3 and 4. Four pairs of biotype 3 (*A"a*) were found that came true in F_3 and subsequent generations when inbred, while the biotype 4 of F_2, only 1 pair gave progeny, and these were of biotype 4 and biotype 2. In F_3 the biotype 4 showed 3 pairs out of 11 breeding true in F_4 and F_5, but in F_5 one pair again gave biotype 2 in small numbers. In F_3 1 pair out of 12 of biotype 2 gave all biotype 2.

From the mating B in F_2, 1 pair, all that gave progeny, continued to breed true through F_3, F_4, F_5, and F_6. Mating C of biotype 5 was heterozygous, 5 pairs giving in F_3 two classes: biotype 5 (*C"*) and biotype 9 (*C'*). Matings in F_3 gave of biotype 9 only one pair with progeny, all of biotype 9, which continued to breed true in F_4, F_5, F_6, and F_7. Three matings of biotype 5 were found to breed true *C"a*, and came true in F_4, F_5, F_6, F_7, F_8, and F_9, while 10 pairs did not breed true in F_3, but were not analyzed further (*C"b*).

One might continue this type of analysis without limit. From the natural population of *L. multitæniata* at any point in its geographic range, materials taken from nature show this diversity of gametic constitution as regards this or any other character that might be considered. It follows that each individual may be different from every other in the population, even in a single character, and any individual from nature may produce many geno-different gametes. It has been clearly shown by Johannsen, Jennings, and others that in self-fertilized or sexually reproduced forms any genotypic constitution that may be selected by the observer can be made to continue in straight lines, but in sexually reproducing types it is these " biotypes," or groups of like gametic make-up, that are to be looked for, isolated, and studied.

A considerable number of writers has isolated genotypes and biotypes that breed true and are asserted, especially by Jennings, to be incapable of modification by cumulative selection. Jennings, in *Paramœcium*, worked only with clones, which are not exactly comparable to genotypes of self-fertilized organisms, and not at all to biotypes of cross-fertilizing forms.

In almost all organisms genotypes and clones will be absent and the biotype will be the group mostly studied and employed. Johannsen's clear definition that a biotype is a group of individuals possessing the same germinal constitution (identical genotypically) leaves no opportunity for misconception regarding the nature of biotypes. A current idea among the supporters of the pure-line hypothesis is that genotypically different gametes have no intergrades between them, but are discontinuous. Woltereck, working with *Daphnia,* has denied this, and others from less secure data have also criticised the view.

Two questions seem of fundamental importance in this connection and involve the validity of the entire conception of minor groups within the " species."

(1) Where in any instance does the limit lie with respect to the determination of a genotypical constitution? I can with my materials, as shown in the first part of this section, easily isolate a race that is *homozygous in behavior,* absolutely so, which breeds true without limit to the one type, and is in every

way a typical group of individuals of like gametic constitution—a biotype. This character of the pronotum is composed of many elements, each capable of independent expression, and each to a greater or less extent independently capable of modification. In figure 41 are shown certain true-breeding races, homozygous in action, with patterns that are capable of having their elements modified greatly, without changing the homozygous nature of the pattern as a whole. Are there biotypes within biotypes? If so, where is the limit? Would it not be possible to go on indefinitely, as skill and methods become acute and refined, more and more to get *ad infinitum* smaller and smaller genes and geno-differences, until a stage is reached in the distant future where the only difference was one of presence or absence of one electron or other minute material unit?

(2) Do these groups—biotypes or aggregations of genotypically like individuals—exist in nature as isolated realities in the population, or are they merely individual momentary conditions present in members of the population which can be separated and when isolated breed true as long as the isolation is maintained? What is the position and meaning of these experimentally isolated groups in populations from nature.

There is no doubt that from beans, grains, etc., it is possible to isolate genotypes as Johannsen and others have done, or to isolate clones from organisms like *Paramœcium,* as has been accomplished by Jennings; but were all of the genotypes isolated, and how many other genotypes based upon different characters could have been secured? Are we not still thinking and talking of these matters in terms of the morphologist and anatomist? Are not genes and genotypical constitutions at base purely morphological concepts in that they involve identity of parts or constitution? The key to the whole situation lies, I believe, in the determination of what homozygous and heterozygous conditions of the germinal material really signify.

Neo-Mendelians, descriptively at least if not in fact, adhere to the anatomical picture, and homozygous gametes are those of like factorial constitution, and heterozygous gametes those of unlike composition. This is not true. Homozygous as well as heterozygous are descriptive of uniformity of reaction in the germinal material and not of the conditions of constitution *per se.* That is, homozygous gametes are those in which a state of physico-chemical adjustment has been attained, such that, regardless of the active potentialities present, the action of the gamete is always the same in normal development and in combinations. Genotypical gametes are the same in character and action, in that a genotypical gamete or group may be different in their potentiality constitution, but they react alike, behave alike in ordinary breeding, and hence are descriptively stated to be alike. It is a singleness or uniformity of action that determines the behavior, and this is dependent upon the stability of the physico-chemical complex in the germinal material, and not at all upon the nature or array of potentialities present.

The first problem with any natural population is to discover if there are present minor stable groups which can exist without selection to maintain them, but which, nevertheless, interbreed to a greater or less extent in the population. With a species like *L. multitæniata* it is, as has been shown, easy to isolate many true-breeding strains. How many of these will remain without "selection" when carried as group cultures? To what extent can these be modified by cumulative "selection"?

PRIMARY BIOTYPES.

In the population of *L. multitæniata* I have been able to isolate from the pronotal pattern 12 groups that interbreed, overlap, but can be isolated and which remain constant through an indefinite number of generations. These I have called " primary biotypes," and they can be distinguished as follows:

Primary biotype 1—Complete black surface both above and below, without trace of hypodermal color or pattern. (Fig. 35; fig. 36, 1.)

Primary biotype 2—Spots *b'*, *a'*, *am*, *pm*, *a*, *b* and anterior marginal spots forming a solid central triangular area. Spots *c*, *e*, *f*, and *d* and posterior marginal area fused to form an irregular triangular spot in each posterior outer angle. These two larger areas meet along the posterior border in the median line and fuse with the central area through medianward extremes of the *d* spots. This type may vary from complete fusion of all areas to form a central dark area with a lighter margin (fig. 35, 2) to the extreme shown in figure 36, 2.

Primary biotype 3—Differs from No. 2 as follows: The central triangular area is broken anteriorly, traces of anterior marginal spots appearing, and spot *c* is free from the *e-f-g* posterior marginal group. As an extreme *c* may fuse with the lateral group and the anterior spots may by fluctuation give the appearance of a No. 2. Breeding, however, always reveals the real character of the individuals in question.

Primary biotype 4—Differs from No. 2 and No. 3 in that *c* is never united to the lateral fused mass of *d*+*e*+*f*, and the anterior marginal spots are never united to the central triangular area and may be much reduced or even not visible, while the central area becomes more open, showing median separation into two lateral *a*, *b* groups, which are widely divergent anteriorly. The *d*, *e*, *f* posterior marginal area is also reduced, although still connected and fused usually with the *a' b' a b* group by a medianward elongation of *d*, which is deflected cephalward to the anterior end of the *a* areas. This group is variable, as shown in figure 35, 4, and figure 36, 4.

The primary biotypes 1 to 4 overlap and form by their " variations " a graded series, as shown in figure 41, and are distinguished by changes in the relation of the elements of the pattern and not at all by the amount of pigment present.

Primary biotype 5—The central spots form a broadly open V composed of $b'+a' \underset{\times}{\overset{am}{\times}}{}_{pm} \underset{\times}{\times} a+b$. The anterior marginal spots are always entirely wanting on all. The posterior marginal *d*, *e*, *f* are fused and *d* prolonged medianward at 90° to median axis, and fuses with *a* (rarely as a variation; " fluctuation " *e* is prolonged anteriorly to meet *c*). Figure 35, 5, and figure 41, 5, show the character of this biotype. In figure 36 it is shown as forming a diverging line from the meeting-point of the different biotypes and tends to form in appearance a pronotal pattern with a transverse dark band on the posterior half of the pronotum.

Primary biotype 6—Precisely the same as biotype 5, except that the anterior marginal spots are always present in varying degree, as are also the posterior marginal areas. The central group is broadly open, as in No. 5, and united to lateral groups in the same manner. In figure 35, 6, this biotype is shown as another divergent group. The tendency of this group is to form a pattern with the posterior half of the pronotum darkened to a greater or less extent.

Primary biotype 7—Occupies a central position in the series. The central system of spots is always fused into a broadly open V, of which none of the elements ever becomes free. *C* is always free and distinct, and *d, e, f* group are fused but never united to *c* or medianward to the middle group. The anterior marginal spots are always absent and also the posterior marginal. This biotype exhibits as little fluctuation as any excepting 1, and shows only increase or decrease. (Fig. 35, 7.) It is the pivotal pattern type of the whole array, and by adding to or taking from it almost any combination can be built up. It is also a common one in nature.

Primary biotype 8—This comes from biotype 7 by the breaking up of the groups $a'+b' \overset{\times}{\times}\ {}^{am}_{pm}\ \overset{\times}{\times} a+b$ and the d+e+f group into the component centers, figure 35, 8. This is a variable biotype. The anterior marginal spots and posterior marginal spots are never present. *C* is always free and the central and lateral areas never united. Spot *d* may extend anteriorly towards *b*, but rarely fuses therewith.

Primary biotype 9—Shows a sharp change in the position and character of the *a* spots where they are parallel with the median line and not widely divergent anteriorly as in all the preceding types. This is accomplished by the medianward movement of the anterior center in spot *a*, and a cephalward elongation of the spot beyond the *b* spots, giving a more or less squared appearance of the central system. The *d, e, f* group are united and *d* is often fused with *b; e* and *c* send prolongations towards each other, which rarely meet. This results in giving an anterior-posterior striping appearance to the pattern, as shown in figure 35, 9. The anterior and posterior marginals are always absent and there is never lateralward fusion between the central and lateral groups.

Primary biotype 10—Represents a divergent line from 9, in which the same type of pattern persists, but is heavier, the fusion being always marked and heavy, and the anterior marginal spots being present and fused with *a*, forming a prong projecting medianward. This type is represented in figure 35, 10, as a quite distinct but not common one in the population.

Primary biotype 11—Differs from biotypes 9 and 10 by the absence of all marginal spots, the fact that *a, b,* and *c* never fuse, and the tendency to fuse between *d* and *e*, which do not fuse with either *a, b,* or *c*. (Fig. 35, 11.)

Primary biotype 12—This is comparable to No. 8, in that it represents a case in which all centers are free, the only fusion being produced by somatic variations. A tendency to restrict the pigment formation to the color centers also accompanies this biotype. Figure 35, 12, shows the usual range of variation of this group.

These 12 biotypes of the pronotal pattern are based, as has appeared in the above description, upon a system of color centers shown in figure 40, between which certain combinations are made, and to the occurrence of certain marginal spots which enter into the combinations.

These biotypes overlap in their " variations," giving a complete intergrading series in any population, and all interbreed freely when present, so that the natural population is exceedingly complex with respect to this character. These *primary biotypes* may easily be entirely isolated and manipulated as " units," but are to a greater or less extent associated with other characters or groups, form-

ing a larger biotypic group. Thus No. 1 is in nature usually associated with the *melanothorax* form, broad and rounded, while the biotypes 9, 10, 11, and 12 are always present in and correlated with the appearance of the *multilineata* form-factor. Biotypes 2 to 8 are found associated in the species with the form and body characters common to *multitæniata.* In any population there are thus complicating factors in any character, so that a complete analysis would be an analysis of all characters and their interrelations. The pronotal pattern, however, is sufficiently independent and so accurately a gametically determined character that it serves well for certain purposes of investigation.

To recognize these biotypes is of little or no use unless they be tested fully with regard to their properties and capacities for existence. Can such groups exist in freely-breeding cultures without change? Can they be selectively changed by quantitative selection without adding something to the complex? What is the relation of the different groups when crossed? These are some of the determinations that must be made concerning the nature of these biotypes.

THE BREEDING OF PRIMARY BIOTYPES SELECTION.

When one of these biotypes has been isolated by any process, can it stand alone, isolated from intercrossing, without constant selection? This I have tested as follows: Biotypes extracted as shown in this section were started from 1, 2, 3, or more pairs of like, homozygous-extracted types. These were either the progeny from a single pair or from one or more pairs, and were allowed to inbreed and continued to test the stability of the type.

The simplest to deal with is the type 1, and this I have reared in such cultures many times. A pair of F_7 *A'* primary biotype 1 were taken from the culture shown in figure 37 and allowed to breed. They gave 97 progeny, 47 males and 50 females, all of the black type, with no variation in pattern. These are not *melanothorax,* but the black thorax transferred onto the *multitæniata* form. Of these, 20 males and 20 females taken at random from the population were allowed to breed freely and gave a progeny of 224 males and 217 females, all of the parent type. From this population, 20 males and 20 females taken at random were again allowed to breed, and these gave in the third generation 196 males and 194 females, all black. This operation I continued until the sixth generation with exactly the same result, when it was discontinued. At no time was there trace of other type or of variation in this line.

An example of the breeding of a primary biotype as a group culture is shown in figure 40, which represents a group culture of biotype 2. The original pair were an extracted stock bred pure in pedigree culture for four generations and came from parents obtained at the Chapultepec colony. 4 males and 4 females, 2 of each of the kind shown, were mated and these gave a progeny (F_1) of 64 males and 80 females, distributed as shown in figure 40, on the scale of the range in this biotype shown in figure 35, 2. From these 3 males and 3 females from each class represented were taken at random and again inbred and gave a progeny (F_2) of 183 males and 195 females, distributed between the two main classes. 3 males and 3 females from each of these classes were taken at random, mated, and gave a progeny (F_3) of 200 males and 197 females, distributed in four classes, as shown. From these, 3 of each class were taken at random, as far as possible, mated as the parents of generation 4, and gave a progeny of 369 males and 360

females, distributed in the four classes common to this biotype, and one group of 6 males and 1 female having some of the characters of primary biotype 3 (see fig. 40*e*). These divergent individuals were mated, the single male and a female, and gave a progeny in F_5 of 21 males and 26 females, which were, however, all of the primary biotype 2 character. From the normal group in F_4, 2 of each sex were taken at random from each of the classes, mated as the parents of F_5, and gave a progeny of 260 males and 274 females, with the usual distribution. In F_5 each of the two groups were bred from and both gave in F_6 from the normal 131 males and 92 females. In the group B, 36 males and 35 females, all in both sets typical of the strain. The lines A and B were continued, giving in F_7 in (A) 233 males, 191 females, (B) 65 males and 60 females, all true to type, and in F_8 (A) gave 248 males and 279 females, (B) 131 males and 123 females, likewise true to type. At the end of F_8 the culture was killed off.

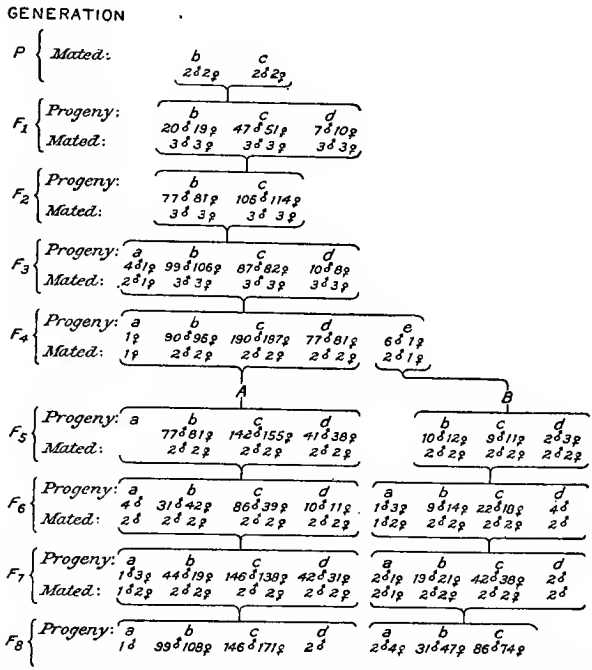


FIG. 40.—Showing breeding record of a primary biotype 2 of *L. multitæniata* through a series of generations and the constancy of the pattern produced in these biotypic lines.

In this test with primary biotype 2, when reared as a group culture with matings at random and perfectly free interbreeding of the parent groups, the culture remained stable throughout. Only in generation 4 did the population overstep the normal range, and these, separated and reared independently, proved not to be a gametic change, but only a somatic (?) disturbance, as shown by the giving of typical biotype 2 pattern all through the line (B), with no reappearance of the divergent examples of generation 4. In a culture of this character there is no intentional selection and no evidence of selection of any sort. The conditions of life were kept as uniform as possible and care was

taken not to allow anything to happen, such as sudden changes of the conditions or violent stimuli which might upset the constitution of some gametes and thus introduce another factor and complicate the results.

Another example will suffice to show the manner of reaction of these biotypes to these breeding-tests in the testing of biotype 9, as shown in figure 41. Two males and two females, each from classes a and b of this biotype, that had been derived from extracted stocks from the Texcoco colony and had been pedigreed for four generations, were mated and allowed to breed freely and gave in F_1 a progeny of 87 males and 101 females, distributed over the four classes common

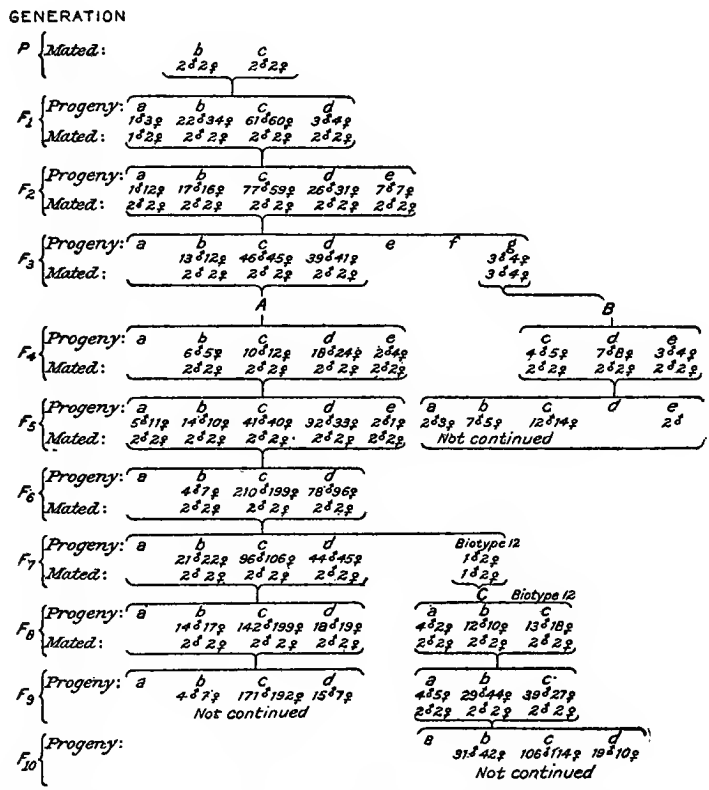


FIG. 41.—Breeding record through a series of generations of a race of primary biotype 9 in *L. multitæniata*, again showing the constancy of the race within this line.

to this biotype, as shown in figure 41. From these there were taken 2 males and 2 females from each class, except class a, and mated. This group gave a progeny of 136 males and 124 females in F_2. This population showed a group of 7 males and 7 females, which were quite beyond the normal range of this biotype; they were not removed, but were bred back into the population in the mating for F_3. From this population the usual random matings were made and they gave in F_3 101 males and 102 females. In this population 3 males and 4 females stood quite without the range of the normal biotype and were separated

and inbred as division B, division A being the normal culture. In F_4 the normal group (A) from a routine mating gave 36 males and 45 females, while all of the progeny of the divergent B group were entirely of the normal type arrayed in classes c, d, and e, there being 14 males and 17 females. From these F_4 groups matings were made as before from A and B, and in both normal progeny resulted in A, 94 males and 94 females, and in B, 23 males and 22 females. The B group was not carried any longer, while the A group continued from the usual mating, giving F_6 all normal, 292 males and 282 females. From these a mating of the ordinary type gave F_7 with two groups, the normal (A) and a divergent one (C) well outside the normal range. In the first there were 164 males and 166 females, and in the second, 1 male and 2 females. A mating of the first group gave normal progeny in F_8, and again in F_9, when the A line was terminated. The C group gave a progeny in F_8 all of primary biotype 12, 29 males and 30 females, distributed in classes a, b, and c. A mating from these gave F_9, again all of type 12, 67 males and 76 females, and from a mating taken from these a progeny was reared in F_{10}, again all of type 12, 156 males and 166 females. At the end of F_{10} this line was also discontinued.

This culture of biotype 9 shows two common types of behavior that are found in these groups, namely, individuals that appear beyond the normal range but which, when inbred, give the normal population, and a group (c) beyond the normal range and without intergrades, which, when inbred, give individuals true to their own type, which continued to breed true to that type. That is, in the language of the adherents of the hypothesis of pure lines, biotype 9 had "mutated," giving biotype 12. The individuals of the group c, however, were the last to emerge; indeed, it was thought that all that were going to emerge had done so and the cages were therefore set aside before being cleared for the next mating. In this period of over a week they dried completely and were often heated to nearly 100°F. The modified group (*c*) emerged after this treatment. Whether the treatment and the divergent group are cause and effect is a matter of opinion. It seems plausible to think they are, but the test is too crude to be more than a plausibility. This occurrence does, however, show one point of importance—the delicacy of balance of some of these groups and the ease with which incident forces may possibly give rise to sudden changes, "mutations," to other biotypes.

Each one of the biotypes shown in figure 35 has been reared in this same manner many times with uniform results. They are groups which, under uniform conditions, are permanent and stable even in unselected group cultures. Under uniform conditions and isolated they remain true to type, fluctuating within a fairly normal and fixed range, but in all of them there is considerable unstability manifested in the presence of extreme conditions or of intense stimuli, or even of rapidly varying conditions, giving as a result sudden changes of a few individuals to other biotype groups.

In this array there are also differences as regards their stability. Biotype 1 is highly stable and difficult of disarrangement, while biotype 7 is the least stable of all. Biotypes 5, 6, and 10 are fairly stable, while between 9, 11 and 12, and 2, 3, and 4 sudden transmutations are common and easily produced, and the conviction is soon forced upon the observer that these groups, although distinct and stable under constant conditions, realities in every respect, would nevertheless under natural conditions present a never-ending series of transmutations as the series of external forces passed over the population.

As far as the continuity of this group is concerned and their gametic identity of constitution, I am fully agreed with Johannsen and others that they are capable of isolation, are *analytical realities,* and breed true under constant conditions, and further, that they frequently mutate to other biotypes within the general population. I am not convinced that they are stable, elemental units in the species, as many consider them. They represent more or less stable combinations attained in the gametic complex, and are but momentary pauses in the ceaseless metathetic recombinations which each organic group is constantly undergoing. That these biotypes are not capable of modification, or are capable of modification only by sudden change, is true for some, not true for all, and in the next section I shall show how these biotypes can be changed by different agencies.

THE MODIFICATION OF PRIMARY BIOTYPES.

In the attempt to modify any of these minor groups, be it genotype, clone, or biotype, an hereditary historic influence suggests selection and external conditions, and when these have been adequately or inadequately tested, other possibilities may then receive attention. Again and again have workers with pure lines, clones, genotypes, or biotypes recorded their inability to produce changes in the types they had isolated. No difficulty is encountered in discovering and isolating races that are alike in most of their characters, but differ in one essential character which sets it off, and when reared in " pure lines " keep it distinct from its fellows. Nevertheless, no effort seems to be able to modify this into another one.

Jennings, in his masterly studies in *Paramœcium,* where sexually produced " clones " were used, finds that he was unable to modify or transmute any of his " clones " into another, either new or existing. Johannsen and others in beans, Nillson-Ehle with grains, East, Shull, Emmerson, working with genotypes and biotypes, found difficulty where modification was attempted. Many workers using phenotypes or elementary species, as De Vries, have found difficulty in modifying them. Why this difficulty? Is it real, a limitation upon one's experimental ability, or is it due to lack of skill and understanding?

The attempt to modify these groups by so-called selective methods seems an indifferent characterization of what one has tried to do, and the word " selection " implies a choice between two or more possibilities rather than a chance determination of what happens. This selective problem is a large one and is one upon which I shall wish to dwell at length later on. I have used, as methods of attempting modification, accumulation of pigment surface in the pronotum as a whole; accentuation of directions of variation in the elements of the pattern (particular spots); synthetic combination of directions of variation; external incident climatic forces; and food and metabolic disturbances.

EFFECTS OF QUANTITATIVE ACCUMULATION OF PIGMENT.

Almost all selection investigations degenerate into the effort to accumulate the same quality or attribute by quantitative additions *ad infinitum.* The odd idea that existing strange modifications of supposed adaptions in nature have arisen by continuous accumulations of minute amounts of the quality lies at the base of this experimental effort. It is notorious that this quantitative accumulation soon reaches a limit beyond which the modification does not extend. This limit in the

huge majority of cases corresponds to the upper limits of the natural "variation" of the species, does not transcend the natural variability, although a fairly stable race is often capable of being created at this upper limit or at any other point by this process.

Of late years it has become the custom to interpret any such result on the assumption that the race obtained is an isolated genotype or biotype. Is this the truth of the matter? It is the experience of so many observers that these selected races revert to the parental standard when selection has ceased that there must be some foundation in truth, and it must represent some result of natural operations. Isolated genotypes or biotypes are asserted not to change, to be difficult of modification, and Jennings says no one has thus far been able to modify one of these isolated groups into another. It is not likely that intercrossing with other biotypes is solely responsible for this regression, and why should the "selected" group so constantly move back to the mediocre of the entire population? Why this submergence of an improved, "efficient" (?) race by the mediocre.

In the population of *L. multitæniata* I have shown that biotypes of the pronotal color-pattern are present, have been separated, and the distinguishing characters of these have been given. In the general population I can easily create a race at any of the extremes of the natural variability by the use of quantitative accumulation. The relation of this to the biotype question is not as simple as one might suppose and will be considered later. In the isolated biotype, what can quantitative accumulation accomplish?

A good test of this can be made by starting with biotype 4 and trying to accumulate pigment and produce an end-result like biotype 1. Three sources of error must be rigorously eliminated: (1) it must be certain that the biotype 4 is pure, contains only biotype 4; (2) the culture must be kept under uniform conditions to prevent any action of external agents upon the pattern; (3) the choice must be made upon the amount of pigment, and no attention paid to the fluctuations in the pattern type. The problem is, can accumulation of pigment or any other character produce directly or indirectly a change of type? I have made many tests of this kind, of which a good example is the following, wherein the attempt was made to change biotype 4 into biotype 1.

Start was made upon a pure extracted biotype 4 stock derived from parents which were obtained in nature at Chapultepec, and which in F_2 gave only biotypes 4, 5, and 6. In F_2 pure-breeding examples (homozygous) of biotype 4 were found and these were carefully pedigreed through F_3, F_4, F_5, and F_6, and from the latter generation the basis of this culture came, while the stock was continued until F_{10}, when it was accidentally lost. It did not show any divergent individuals or tendency to be transmuted into any other type.

Three males and three females of normal average aspect were mated, and gave a progeny, F_1, of 143, distributed as shown in figure 42. From these, 4 males and 4 females of the most extreme class were mated and gave a progeny of 79 individuals, with the distribution shown in figure 42, of which the upper range was above the normal range of the biotype. In F_2, F_3, and F_4 the same method was followed of mating from the extreme upper class, and this rapidly caused the movement of the population towards a condition of extreme pigmentation, until in F_5 the upper limit had reached a state just below that of biotype 1.

From F_5 to F_{10} the same method of mating and selection of parents was followed, giving a race of constantly decreasing variability, and, as far as amount of pigment was concerned, much above that of the normal parent strain. In F_7 there appeared 1 male entirely black above on the pronotum, but not on the sides. This individual was separated and tested separately.

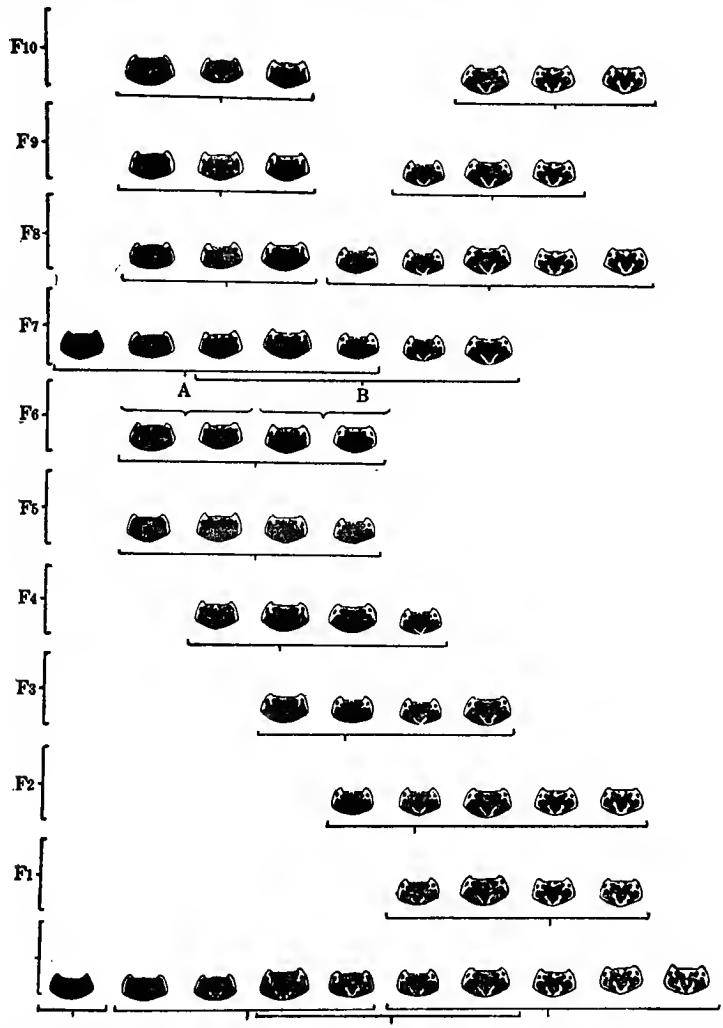


FIG. 42.—Diagrammatic representation of the effort to modify through accumulative selection the amount of pigment in a pure extracted biotype 4 stock of *L. multitæniata*. The figure shows graphically the ineffectiveness of quantitative selection in permanently altering the type of this race.

In F_8 two matings were made; one (A) continued the intensely selected strain through to F_{10}; the other (B) was a random mating from all classes and was allowed to inbreed freely. Figure 42 shows, without further comment, the result. The selected race was kept up to the standard, the nonselected at once began to move backwards and soon showed only the normal biotype 4 pattern.

In F_5 there were 4 with almost totally black pronotum, or biotype 2 form, and in F_5 and later such were constantly present in the selected strain. Superficial examination of the culture would at once decide that biotype 4 had been changed

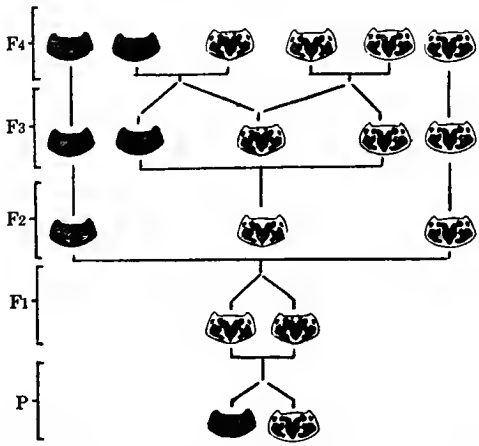


FIG. 43.—Graphic representation of a normal cross between a true homozygous acting biotype 1 and biotype 4 in *L. multitæniata*.

to biotype 2, and the single male in F_7 would ordinarily be interpreted as evidence of the modification to biotype 1. This I tested out as follows: If the extreme male of F_7 is a biotype 1, or even partly so, breeding would test it fully

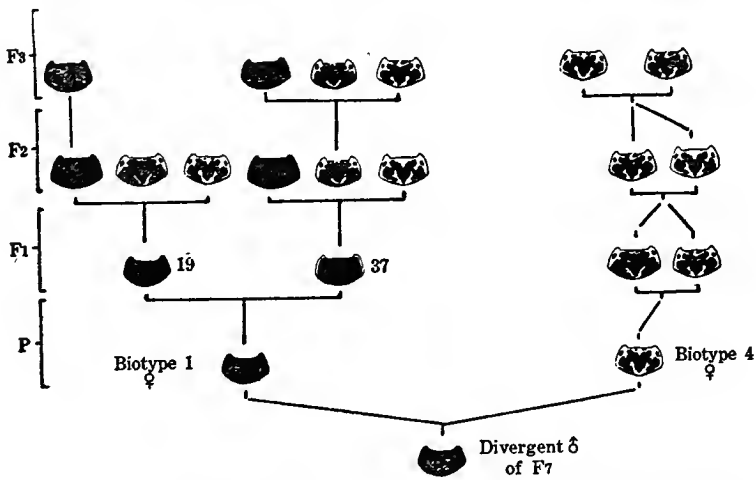


FIG. 44.—Graphic representation of a test between a selectively divergent male of biotype 4 population, shown in figure 42, in which a divergent male was crossed with an homozygous female of biotype 1 and biotype 4. The test shows clearly that the divergent male was distinctly a normal biotype 4 and had not been altered selectively in any respect.

as follows: This male was first mated with a pure female type 1, and second with a pure female type 4 of another strain. At the same time there were mated four cultures in reciprocally mated pairs from the two test strains of biotypes 1

and 4; all gave progeny, and in all the expected results were obtained. Each test strain behaved as a homozygous line, giving in F_1 an intermediate between biotypes 1 and 4, and these in F_2 segregated into the three well-defined groups shown in figure 43. F_2 mating in 8 pairs gave exactly the expected results of segregation and permanency and in close approximation to expected ratios.

The extreme male in this experiment in F_7 was kept a day or two with the female of biotype 1, and then an equal period with the female of type 4, and this repeated as long as the pairs continued breeding. In this way any error from an assumed influence of age, strength, environment, or the determination of anything by these factors is eliminated, as both lines A and B were served alike by the divergent male. In the cross with the biotype 1 female and F_1 population were black, or black with a narrow margin of light color, 19 of the former to 37 of the latter. Six matings from each of these groups, all giving progeny, gave in all identical results, a very ordinary behavior in F_2, with segregation into blacks of biotype 1, clearly marked biotype 4, and intermediate types which were heterozygous, as shown by the breeding of F_3 and shown in figure 44. In F_2 the totals from the twelve pairs is shown in table 34, which was a fair approximation to a normal ratio, although biotype 1 in all sets of progeny was slightly in the lead in point of numbers.

TABLE 34.

	Biotype 1.	Heterozygous.	Biotype 4.
Observed	553	945	451
Expected	447.25	974.5	447.25

The mating B with the biotype 4 female gave quite different results. In F_1 a variable progeny, largely like type 4 in character, resulted, and of these 10 pairs were mated, and all gave in F_2 like results, and all gave a rather invariable biotype 4 progeny in F_3 with low amounts of pigmentation; of these another 10 pairs of F_3 mated at random from the population gave in F_4 another population of the biotype 4. This result is also shown in figure 44.

The result of this test of this divergent black male is in every respect to show in an indubitable manner that, irrespective of its superficial aspects, it was not modified at all as far as its homozygous biotype 4 nature was concerned. It was black over the entire surface, but its gametes were not of biotype 1, but were still unmodified biotype 4. No discoverable effect of the quantitative accumulation of pigment was found and every chance was given this extreme male to show its capacities.

A second series of tests was made of this selected race in F_9, where matings of extreme individuals were made in reciprocal crosses with a pure-breeding, homozygous-acting strain of biotype 12. In this cross other characters of alternative behavior are involved—*i. e.*, body-form, elytral color, etc. These are, however, not considered here—only the array presented of the particular pair of contrasted characters—*i. e.*, selectively modified biotype 4 and homozygous-

acting biotype 12. In figure 45 I have shown the behavior when biotypes 10 and 12, both from homozygous-acting extracted strains, are crossed. In F_1, uniformly, type 4 is completely dominant and in F_2 there is segregation, usually into three classes, on the average of 1 : 2 : 1, as far as this pair of characters is concerned.

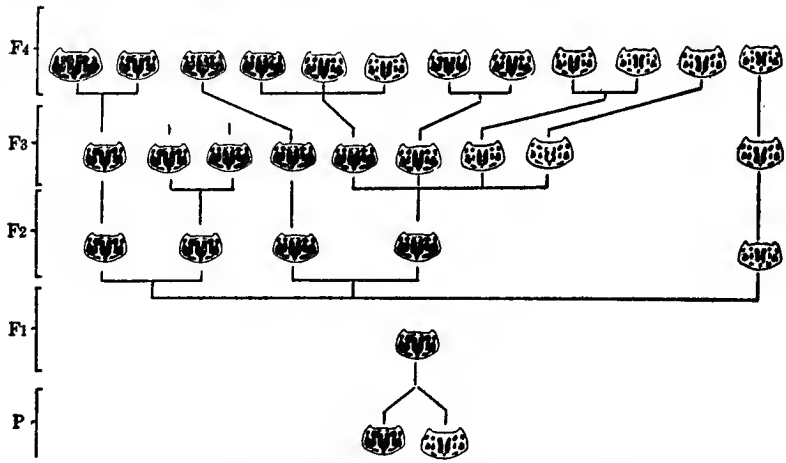


FIG. 45.—A second test made with respect to the same modified character as shown in figure 42.

In the test (fig. 46) 6 reciprocal pairs were mated between F_9 of the modified biotype 4 and biotype 12, and gave an F_1 population all alike with biotype 4 dominant, 247 in all. Of these, 10 pairs mated gave progeny, an F_2 generation in three classes, variable biotypes 4, 12, and typical biotype 4 nonvariable. Inbreeding each class in F_3 showed the type 12 were true-breeding extractives

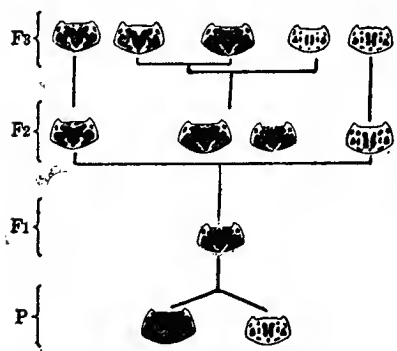


FIG. 46.—Graphic representation of a further test between a selectively modified race crossed with biotype 12, showing that it was not modified but was the normal biotype 4 as far as its gametic composition was concerned.

in the 7 pairs tested, and that the biotype 4 nonvariable were pure, while variable biotype 4 were heterozygous dominants. At no point in these tests have any indications of gametic alteration in the biotype 4 as the result of the quantitative accumulation of pigmentation been observed.

In this entire experiment and its testing great care was exercised to keep out extraneous factors which might vitiate the series. At no time has there been any indication of gametic change. How, therefore, are these changes, often beyond

the normal range as far as operation is concerned, to be interpreted? This intense "quantitative accumulation" of a character did not in any way change the gametic nature of the strain. Only in a certain portion of the strain there was an accentuated condition of the organism, such that pigment was produced over a wider area of the surface than was usual in the race experimented with. The result is comparable to the quantitative accumulation of any other substance or condition in organisms which may have been recorded. In the experiment described it is clear that increased amount is persistent through many generations, so long as only parents with high pigment-forming values are used as the progenitors of the next population, and it is evident that some germinal constituent is exaggerated, but in what respect? The pigment itself is passive, a product of the oxidation of a chromogen, and a capacity for pigment production exists at all times in all portions of the organism. Whatever determines pattern, either itself has, or has associated with it, something which inhibits pigment formation over certain areas and allows it in others. In the selective accumulation *that factor which limits pigmentation in the normal strain* is weakened so that it allows pigment to be formed over wider areas than is normal, but it appears to be impossible to do more than diminish (or increase) this factor by the cumulative method, and no fundamental change is made either in the factors present or in their relations, and only a distorted action (a condition) is produced which may be varied at will.

Another series of experiments similar in import was made by starting with biotype 4 and attempting, by quantitative diminution, to produce biotype 8 in its extreme form. For this material from the same culture of homozygous-acting biotype 4 stock was taken and matings were made from the extreme lower limit of the type present. The results of this attempt are shown in figure 47. No modification, no change of pattern, and little decrease of pigment was found, and the whole culture presented the appearance of being against a limit below which it was not able to be forced by mere subtraction of substance.

At different times I have attempted modification of other biotypes in *L. multitæniata, but never with any success when the method of quantitative accumulation or diminution was used.* The continued attempts to produce changes by this method have thus far resulted in failure by all workers and in all kinds of organisms. The reason for the failures appears to be because there is nothing in the method of operation which could either remove any factor or change its relation in the germinal complex. These biotype groups provide adequately refined materials for such tests, and while with complex populations (mixed) results of supposed quantitative accumulation may be obtained, the results are due entirely to other processes. In refined strains such as I have used or as have been used by Jennings, Johannsen, and others, when properly protected from errors due to improper experimental culture, results of the same kind have uniformly been secured.

The results show that there is in the method employed no point of entrance produced in the organic system whereby anything can be added to or taken away, or any relation within changed, and these are the only known means of inducing change. In the strains the continued homozygous-acting gametes give in each reproduction and in gametogenesis the same processes over and over again, with only increase or decrease of some factorial action and manifestation. There is no opportunity for metathesis or for possible change following this. The

inefficiency of the quantitative accumulation method is because it is without a physical means of producing real change in any refined homozygous-acting population, regardless of whether it is large or small. Whether genotype, biotype, phenotype, or species, so long as the homozygous action of the gametes is maintained, no quantitative accumulation can become effective for change of any kind, and this is the real reason why quantitative accumulation is inefficient in organic transmutation, and is not an agent in evolution.

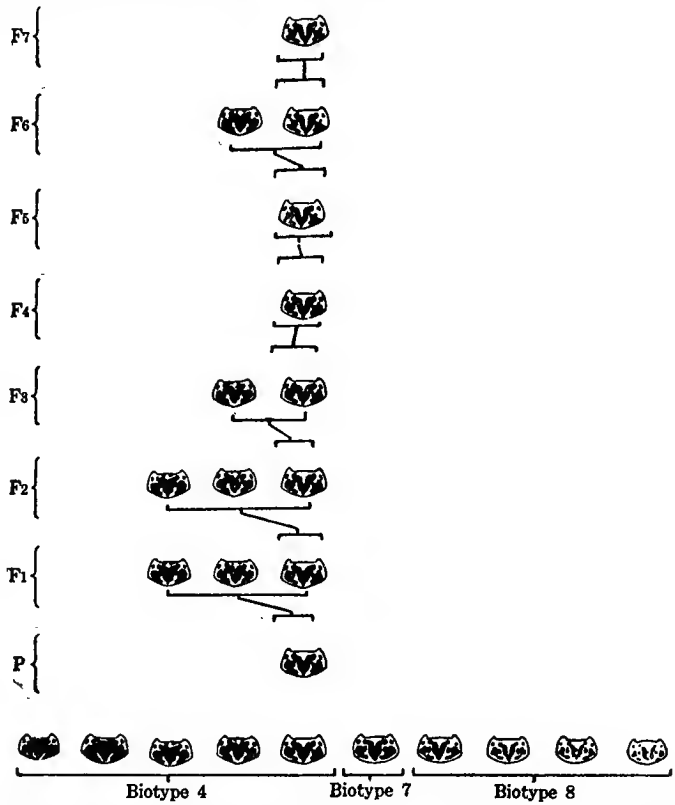


FIG. 47.—Graphic representation of the effort to reduce the amount of pigment and thus alter the character of biotype 4 in *L. multitæniata*. No effect was produced.

The far-reaching and fundamental truth of this only becomes apparent when one is dealing with pure, homozygous-acting, gametic constitutions, and there the reason for its inefficiency appears. With impure lines " the method " will no doubt be in the future asserted to be efficient and productive of results, which in reality are due to other agencies acting in heterogeneous material. There is no doubt that quantitative accumulation plays a rôle in many operations of change, but not directly, as I shall show in other portions of this report. Indirectly it may be still an active agent in evolution in mixed populations, but with pure materials in organisms and in non-organized matter no effective means is provided whereby quantitative accumulation can add new, take away, or produce rearrangements independent of other more active and effective agents as incident forces and the interaction of the prime factors of constitution.

EFFECTS OF QUANTITATIVE ACCENTUATION OF DIRECTION OF VARIATION IN ELEMENTS OF PATTERN.

In this application of the idea of modification through quantitative accumulation or subtraction I shall deal with changes involving only the directions of variation in simplest characters, the present indivisible elements of the pronotal pattern. It has been shown in an earlier section how the elements of the pronotal pattern "vary" in different directions, and how this results in the production of quite diverse combinations or patterns. It has further been shown that the biotype groups are mainly dependent upon this difference in combination of the elements into stable, separated groups, capable of isolation by modern methods. In this section is considered the possibility of modifying quantitatively the fluctuations in the lines of variation present in the simplest characters.

The problem can be attacked by attempting to change biotype 4 into biotype 5, by the attempt to change the direction of fusions between (a) and (d) from one in an antero-mediad direction to the anterior end of (a), to a strictly transverse direction as in biotype 5, and at the same time obtain by selective reduction a loss of the anterior and posterior marginal spots of biotype 4. In these experiments only homozygous-acting, carefully pedigreed stocks, reared under uniform conditions, can be used, and any deviation introduces errors that obscure or falsify the results.

Many attempts have been made to accomplish the result proposed by quantitative change. Thus far no success has rewarded these attempts. At the outset difficulty is encountered in finding the proper fluctuations upon which to work. In many series I have repeatedly failed because the supposed fluctuation in the desired direction proved to be only a somatic disturbance and not at all a gametic change, while others proved to be gametic in character, but of too low capacity for use in accentuation. So, likewise, the attempt to remove the anterior and posterior fusion lines did not succeed, although it was possible to maintain with ease much reduced states of these. In no case did these prove to be wanting in the selected strains. Crosses with these and some other biotype always showed exactly the behavior of a biotype 4 when crossed with the test strains.

The same result has thus far attended my efforts to transmute biotype 7 into either 9, 10, 11, or 12, by quantitative accentuation. The best efforts of which I am capable have been put upon tests of this kind, and I have everywhere been rewarded by complete failure to attain by quantitative change any transmutation from one biotype to another. An example of the difficulty attending the working out of experiments of this type is found in the experiments wherein I was trying to change biotype 7 into either 1, 10, 11, or 12, and tests were made of the real nature of the accentuation shown by crossing back upon another strain of type 7. In 11 instances it happened that there appeared in such tests biotypes of the desired kind, but in all it was found that the test strain had carried in "unseen condition" the pronotal form-factor for the "*multilineata* form," and this crossed with type 7 always by synthetic combination gives fixed pure-breeding stocks in F_2 of biotypes 9, 10, and 11, rarely of 12. The changes found in these tests were not at all a product of quantitative action, but a known result following the presence of a definite factor when introduced into the gametic complex. To those familiar by actual experience with the delicate nature of some of the gametic factorial effects, the above will appear most obvious. It is

only by the elimination of errors of this kind, and of many others, that an adequate test of quantitative accumulation effects can be made. Upon a gross, unrefined population, or unsimplified strain, no such attempt can profitably be made, and any result obtained will be only a change induced by an unknown or at best by a series of two or more interacting forces. Any permanent change, as far as evidence goes, is most probably due to a new gametic state brought about unintentionally by synthetic combination and not to the action of a "selective process."

EFFECTS OF SYNTHETIC COMBINATION OF DIRECTIONS OF VARIATION IN ELEMENTS OF PATTERN.

Quantitative modification has, in every instance thus far investigated, resulted in no change of a gametically homogeneous group, clone, genotype, or biotype, but on the other hand, the effects of intelligent synthetic combinations have given different results. This is due to the fact that in these biotypes a system of elements exists in a pattern system, and between these elements many combinations are possible, but in every instance it requires the concerted interaction of two or more agents to produce a change; that is, one element alone, however changed, can not combine with some other gametically; but it is the experience that both must be involved and show directions of variation towards each other which unite and hold fast the two elements until disrupted by some agency external to the particular group. The existence of these bonds or directions of combination affinities differ in their capacity for permanency in the same type and in different types and species. It has already been shown how these bonds change direction; that their combinations are permanent or transient, and here it remains to show how the biotype groups of the pronotal pattern that can be isolated are also capable of being transmuted by proper treatment. The method of change employed is, in its essential character, organic metathesis, producing interchange and recombination of the characters of the elements of the pattern of the pronotum.

An examination of the pattern of this part reveals the fact that the $b'-a'-a-b$ group is either widely divergent anteriorly in the form of a widely open **V**, or parallel with the b' b at the level of the a' a area. This results entirely from the presence of two form-determiners interacting with a pronotal form-factor. I have shown that in the natural material of *L. multitæniata* there are two general body-shapes which are expressed by the phenotype group names, "*multilineata*" and "*multitæniata.*" Whenever the *multitæniata* factor is present, the spots $b'-a'-a-b$ are a broadly open **V**, and when the *multilineata* factor is present they are closed and parallel. This can be shown as follows:

If a perfectly pure homozygous-acting strain of the form *melanothorax* be obtained, and this combined with the proper forms, tests to prove this above assertion can be made. The *melanothorax* form is another complex; the centers are lost, its ontogeny and everything show it to be different from the rest of the species when in pure strains, even though it is not to be distinguished in the adult condition. If such a pure strain is crossed with a biotype 12, also homozygous in action, there results in F_1 a progeny with the *melanothorax* dominant, either completely or nearly so. These inbred give in F_2 three complex groups—one with the form of *multitæniata,* one intermediate, and one of the *multilineata* form. The proportions are variable, but in the long run are a close approximate

of 1:2:1. Within each of these groups, however, the pronotal pattern shows considerable diversity.

The least numerous groups are those with the narrow form of *multilineata,* and here two types of pronotal pattern occur in about equal frequency—a completely black one and one or more of the biotypes in the 9, 10, 11, and 12 groups.

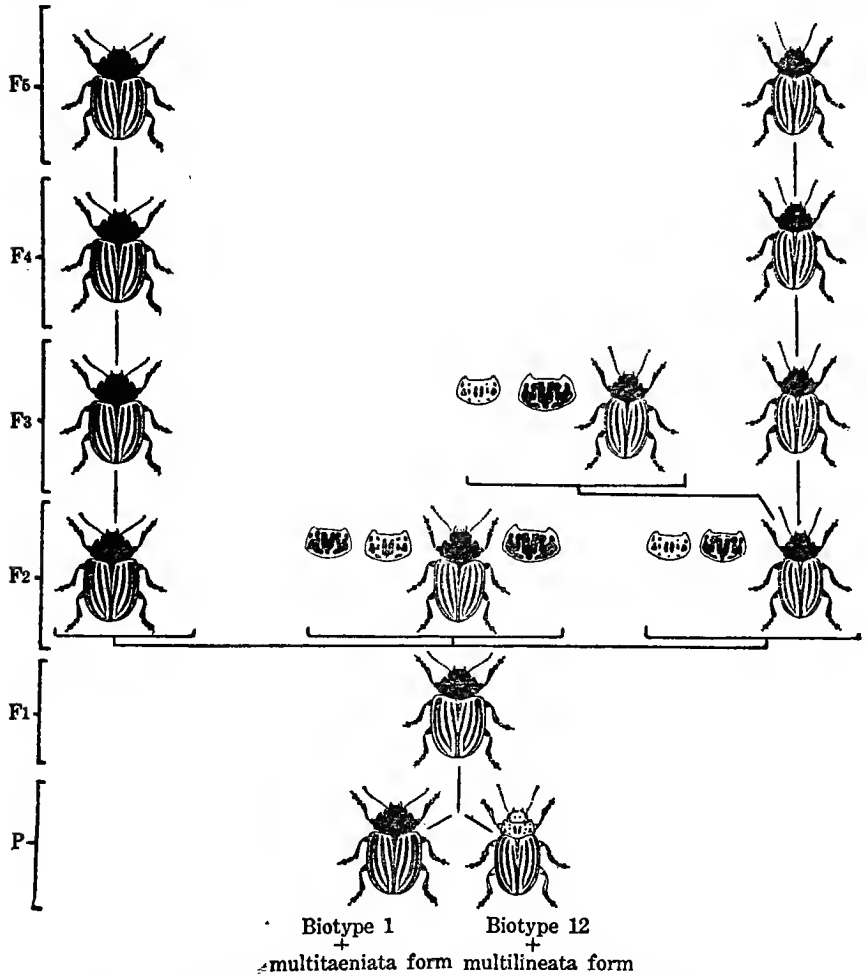


FIG. 48.—Graphic representation of the action of the form-determiner in the alteration of the color pattern of the pronotum.

The second class shows wholly black, while the most common group shows black and a variable array of pattern, as shown in figure 48. In the least numerous group are found blacks that are both homozygous and heterozygous, and from these it is a simple matter to extract a homozygous-acting strain that has the *melanothorax* pronotal pattern in combination with the determiner for *multilineata,* a narrow elongated type. Two test agents, races, are now available, each with the same pronotal complex but associated with the two different form-determiners, and each in pure homozygous-acting biotypes.

One test of the action of these determiners can be made as follows: A race of biotype 4, which has normally the same form-determiner as the normal *melanothorax* type, when crossed with the *melanothorax,* gives an F_1 population variable from dominant type 1 to dominant type 4 (fig. 49). In F_2 always there occurs the one form and three pronotal types—type 4, variable intermediates, and type 1. The first and last are homozygous, the second heterozygous. The only gametic differences contrasted are the pronotal biotypes 1 and 4. If, however, a cross is made between the biotype 4 and the black pronotum plus the *multilineata* form-factor, there results an F_1 progeny variable but of biotype 4 character. In F_2, however, there result again three general groups in form, and each variable as regards pronotal pattern. There appears a group of long, narrow forms—broad, oval, rounded, and intermediates. The ratios of these are variable, but appear to be present as 1:2:4; at least the most numerous group is always the broad, oval *multitæniata* form, the least numerous are the *multilineata* form. It is in this latter that there occurs the evidence of the action of the form-determiner, because here occurs in all instances numerous examples of the biotypes 9, 10, 11, and 12, where *a' b' a b* series are parallel and not a broadly open **V**, as in the other biotypes. Between the two crosses of biotype 4, the only differ-

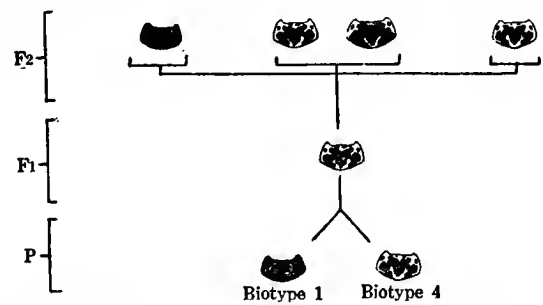


FIG. 49.—A graphic representation of the action of these determiners showing the appearance of pronotal pattern biotypes not present in the original stock.

ence is the presence of the form-determiner, and clearly the presence of the form-determiner acts to change definitely the pattern of this central system of spots and their position, configuration, and interrelations. Its presence has produced, and does produce each time, this definite and exact transmutation, in individuals possessing the factor, the pronotal pattern of either types 9, 10, 11, or 12, or of entire black. There is in this a clear transmutation of biotypes, a transmutation due to a determiner for form, whose presence and action can be experimentally proven. The process which is productive of this change is a synthesis, and recombination following upon an organic metathesis produced by combining unlikes in an interacting complex. This result is shown in figure 50, where biotypes 9, 10, and 11 result, and could by extraction be obtained pure in F_3, F_4, or F_5.

In the same way and by the same method other changes less marked in visible manifestation, but none the less definite and capable of demonstration, can be produced. Thus pure homozygous biotype 3 × biotype 8 will give in F_2 and F_3 homozygous biotype 6 individuals, from which a *de novo* strain of biotype 6 can be obtained. Biotype 8, like biotype 7, is a neutral one with fusions not established and lacking the marginal areas. Biotype 3 carries the marginal centers and the interaction of 8 × 3 gives a transverse system of combinations, as shown

in biotype 6, where the addition of the posterior marginal areas may produce a pattern with the posterior half almost uniformly black. From the same combination there not infrequently results biotype 5, which lacks entirely the marginal areas, but is otherwise like the pattern of biotype 6.

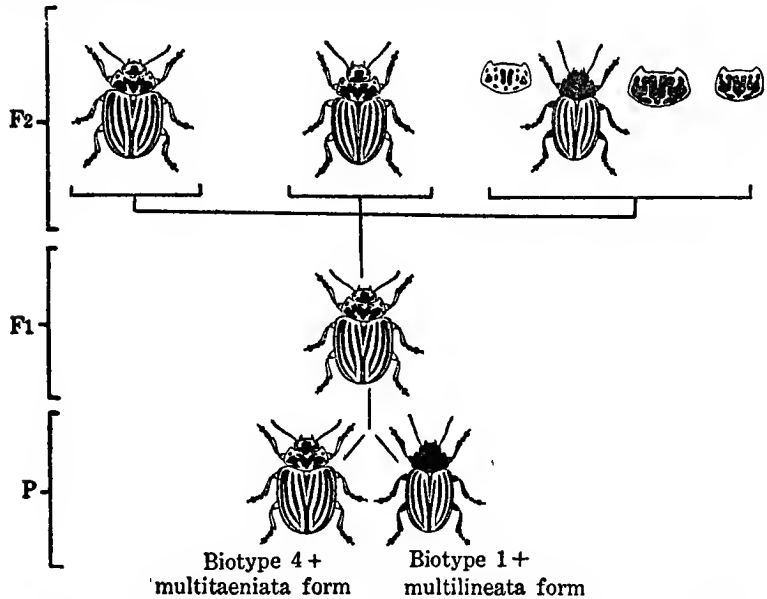


FIG. 50.—Graphic representation of effect of form-factors upon the pronotal pattern type.

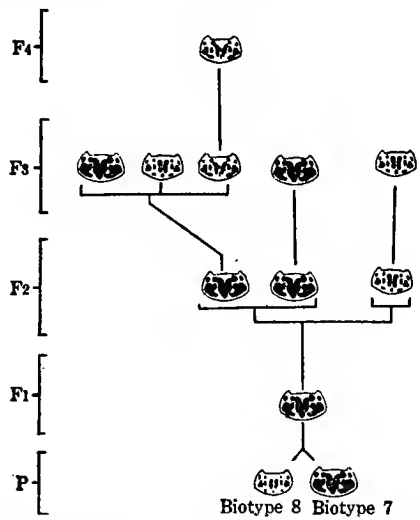


FIG. 51.—Showing effects produced in crossing of biotype 7 with biotype 12 and results produced in the alteration of pattern.

Different results follow when the neutral biotype 7 is combined with biotype 12, which gives as an F_3 extracted biotype 8 (fig. 51,) and in the same way biotype 8 crossed with biotype 9 will give in F_2 and F_3, as an extracted type, the

neutral biotype 7 and biotype 12 (fig. 52). Combination of biotypes 7 and 9 give biotypes 10 and 5 in F_2 and F_3 as extracted forms (fig. 53). This is not the place for an extensive analysis of the gametic constitution and of heredity behavior, and only enough has been presented to show how exactly these "variation" phenomena are a product of gametic constitution, and to show how differing determiners are capable of rearrangement, of extensive manipulation, and are able to transmute one biotype into another by altering, through its presence, the configuration of the pattern complex of the pronotum. The biotypes are realities, capable of independent stable existence; the determiners are no less real, even though unseen, and known only through their action. Not one determiner but several are involved, each more or less independent and capable of altered relations, of being present and acting, or of being entirely removed or made inactive in the complex.

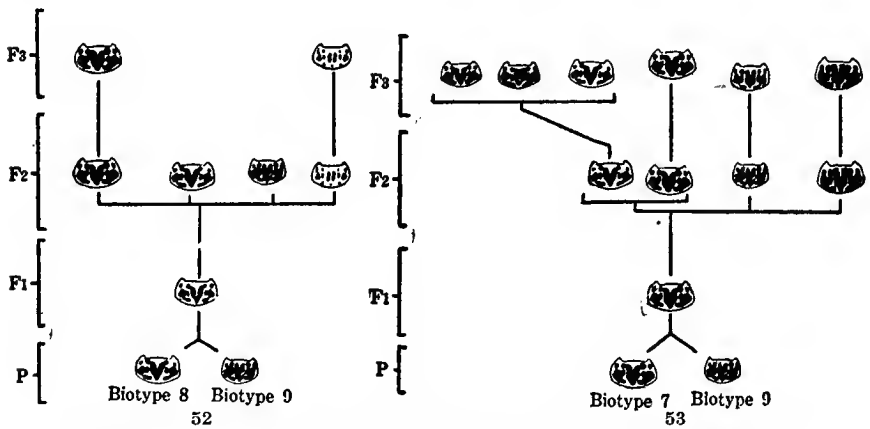


FIG. 52.—Showing behavior of biotypes 8 and 9 when pure homozygous lines are crossed.
FIG. 53.—Showing effect produced in the crossing of biotypes 10 and 5.

In nature there is constant and unlimited mixing of these factors and determiners in all kinds of combinations; but out of this complex of interacting agencies certain definite patterns always come, so that the net result is a rather stable population as far as the patterns presented in any given location. The heterogeneity presented, however, is not one of quantity nor of directions of departure, but is at least analogous to the diversity found in many chemical operations where nearly related compounds are easily transmuted into some other through the presence or absence of something whose presence determines a different configuration of the system, and whose absence permits of another and diverse arrangement. In this pronotal pattern, which is a system as intricate as one can wish, the presence of one or two form-determiners decides the type of the central *a' b' b a* group. The determiner of the posterior and anterior marginal spots likewise determines the resultant changes found in certain biotypes, and so on through the series.

From this point of view the variation in the complex pattern as found in nature presents a very different aspect. It is no longer "variation," but metathesis, and the phenomena must be viewed as purely physical in character

and of chance occurrence, dependent upon chance gametic agents, combinations, and conditions present, as far as their appearance and frequency in nature are concerned.

The discussion and description of the foregoing relations in the pronotal pattern are based upon materials held under uniform conditions of growth and development and provided with optimum food, temperature, and other life relations. In nature the conditions of life are neither constant, uniform, nor optimum, and probably no population ever exists that does not encounter more or less in the way of adverse conditions at some time. It requires but little observation in nature to show that from generation to generation different aspects of the population are presented, and these have usually been attributed to the effects of external conditions. That this is true to some extent no one doubts, but the exact rôle of external forces in any given case has yet to be determined. These pronotal patterns are no exception to the rule, and this aspect of the problem will be considered after a discussion of the conditions in nature have been presented.

CHAPTER IX.

ANALYSIS OF HETEROGENEITY IN THE POPULATION.

It has been shown how the elements of the pattern found upon the pronotum—simplest characters—behave with reference to different factors that are met in the life of the individual. How some of these are related in the complex pattern that is found on this portion of the body and the different aspects of some of the problems of heterogeneity presented by this portion of the organism. There remains the complex character as it is found in nature in the population, the manner in which this system acts as it meets the ever-changing conditions of its habitat, and the metathesis of simplest characters within the population itself. A picture and an analysis of the conditions found will help materially to gain a complete vista of the problems of heterogeneity in a complex character, and help in some measure to aid in the understanding of the causes of this heterogeneity, its limitation, and control within the population.

For this survey of the conditions in the population the tropical forms have afforded the finest material. The species *L. multitæniata* Stål has been particularly favorable for this analysis in nature and for the experimental studies, both in nature and in the laboratory. The fact of its complexity, the fortunate diversity of its natural habitat, and the close proximity of habitats of widely differing conditions have, in the aggregate, produced conditions of uncommon excellence for the proper and successful pursuit of the problems. Naturally the other species in the group have come in for their share of the same treatment, but none of the other species has been given the time or attention that this one has, and none has given the least indication of principles that were in any manner different from those found in the most exploited species. In the presentation of the rather extensive data, the condition found in each colony during the time of observation, with brief comments upon the findings, will be first presented, and at the end a comparison and discussion of the results of the examination and census-taking, after which some of the experimental results will be presented.

IN LEPTINOTARSA MULTITÆNIATA.

THE CHAPULTEPEC COLONY.

The character and general topography of this location have been already described, as well as the methods of taking the observations, and the source of the climatological data used to show the conditions that prevailed in the location as nearly as possible during the time that the observations were being made. It would have been far better if the records could have been made on the exact spot where the organisms were living, but this was not possible, and the records presented show fairly well the general prevailing difference of conditions in this and in the other colonies where observations were made.

The observations were begun in 1904 at this colony and ended at the close of 1909, covering 12 generations in the same location, and are lineal descendant populations, with probably some additions of new blood from outside of the colony. The records show the response of the population as a whole at this location to all of the conditions it had to meet, and as registered on the pattern of the pronotum.

The first census of the population, made in 1904, showed a total count of 3,550 in the colony, with the distribution of the different biotypic groups without breaks, and the array when plotted gave complete gradations along all of the lines with extremes lacking, with the exception of the series composed of biotypes 1, 2, 5, and 10. Comparatively little " somatic " variability in the amount of pigment is shown in the population.

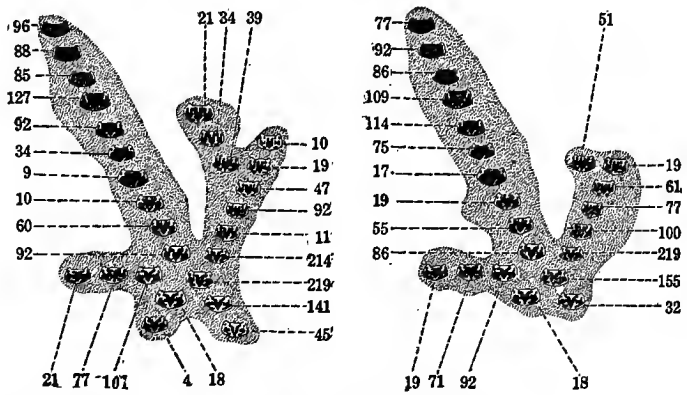
The second census, made in 1904, showed somewhat different conditions, with a population of 1,970 in the colony, and instead of the homogeneous array which the first census presented, the distribution in the second is broken and shows many gaps. In the males there was a clearly isolated group of biotype 1, another of 10 and 12, while the extreme conditions of biotypes 8, 6, and 5 stood apart from the population as distinct groups of considerable strength. The males showed much more variability than did the females where the only distinctly isolated biotype was the extremes of 12. Comparison of the two censuses for the year shows quite different conditions for the population, and illustrates the utter uselessness of a single determination of the condition in the population as a means of discovering the true condition therein. These census records I have shown in figures 54 and 55.

In the third census, made in the early portion of the season of 1905, the population was small in number, 1,897, with a distribution much like that at the beginning of 1904, with no separated groups, and the entire range fairly well represented, with the exception of biotype 12, and the curtailment of the extreme conditions of biotypes 5, 6, and 8.

The fourth census, made in the latter portion of the season of 1905, showed an increase in numbers, 3,230, and the separation of the population into isolated groups, the probable absence of biotypes 5 and 6, the isolation of the extremes of 8 in the males, and the isolation in both sexes of 10 and 11. On the whole, the population in the fourth generation showed much restriction in its range, and this came in a season and the portion thereof when the precipitation was low and distributed in such a manner that the critical periods in the life of the population were passed in relatively dry conditions with high rates of evaporation. The condition in the population for the two censuses of the year is shown in figures 56 and 57, and exhibit about the same condition as in the year 1904, with the absence of extremes, excepting in the series composed of biotypes 1, 2, 3, and 4, and the isolation from the mass of the population of the same groups that were isolated in the second generation of 1904.

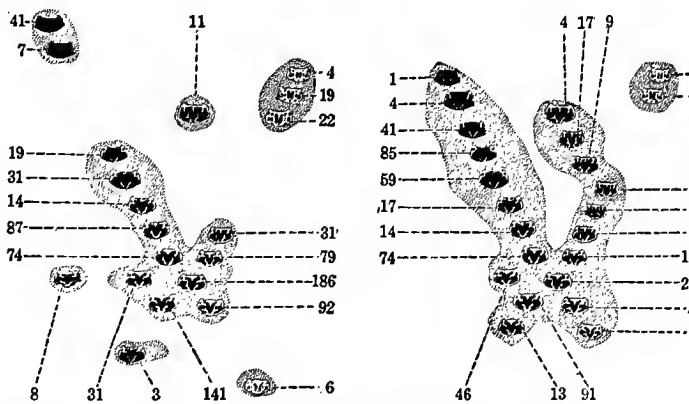
In the year 1906 the first census made (the fifth) showed a new arrangement of the distribution of the population, in that the 9, 11, and 12 series was well developed in both sexes and strong in numbers, whereas the groups 5, 6, and 8 were practically wanting in the population. The series 1, 2, 3, and 4, while present, were, in proportion to the whole, not as strong as in previous censuses. The only isolated group was a small number of males of biotype 5. This condi-

tion in the population occurred in the early portion of the season, when the rainfall was low but well distributed, with uncommonly widely ranging humidity percentages and consequent desiccation during the reproduction of the population that had emerged from hibernation from the previous year.



1705 Males. Total 3349. 1644 Females.

FIG. 54.—Census of first annual generation of 1904 at the Chapultepec colony, showing actual pattern conditions in population and number of individuals in total population showing the pattern observed.

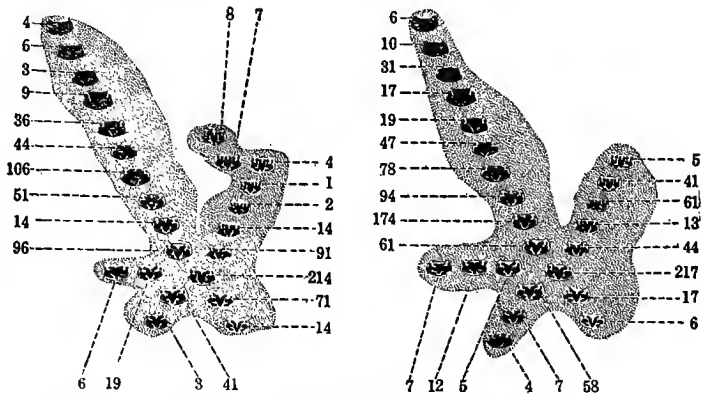


906 Males. Total 1970. 1064 Females.

FIG. 55.—Census of second annual generation at the Chapultepec colony in 1904, showing pattern conditions in the pronotum and the fact that sharp differences in the population may arise between successive generations.

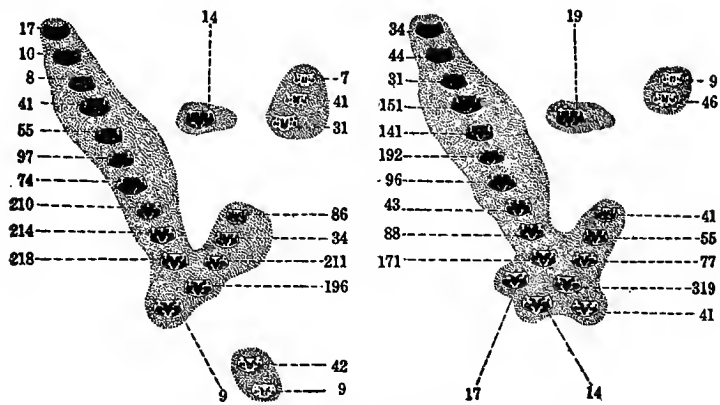
The second generation showed in the census essentially the same condition as in the first for this year. The same groups were present, in about the same strength, but in this generation there were no isolated portions of the population. Biotypes 11 and 12 seem to have been lacking, and in their place 8

appeared in full strength, while 5 and 6 were still practically absent. This generation was the product of the first, but under conditions that were unusual for the place, in that the rains had been light in the earlier portion of the season, the ranges in the humidity had been large, and the desiccation high,



863 Males. Total 1897. 1034 Females.

FIG. 56.—Census of first annual generation at the Chapultepec colony in 1905, showing conditions in the population with respect to pronotal pattern and relative frequency of each type.

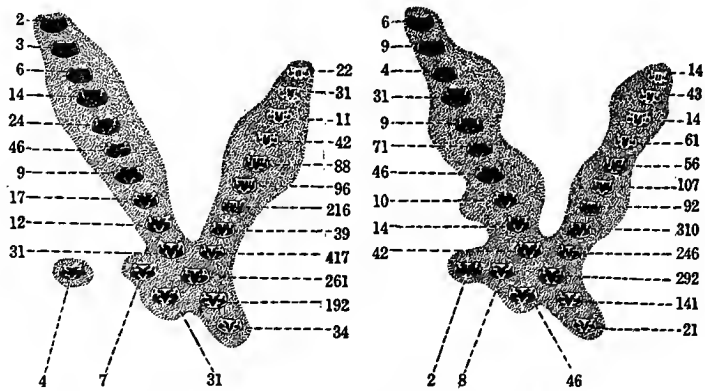


1624 Males. Total 3253. 1629 Females.

FIG. 57.—Census of second annual generation in the Chapultepec colony in 1905, showing the array in the pronotal pattern, and a sudden change in aspect of the population from that shown in preceding censuses.

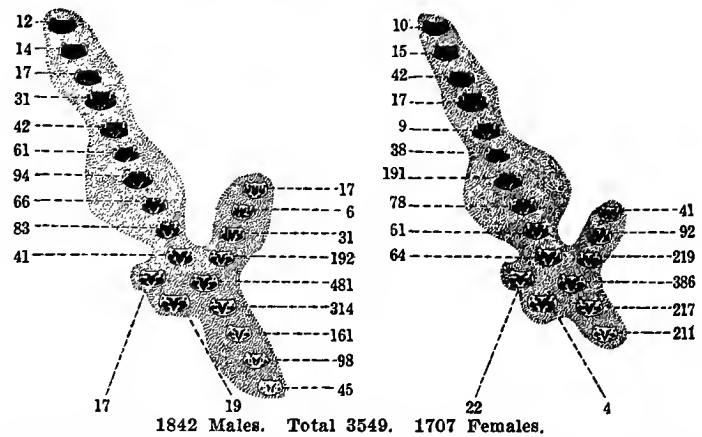
while in the latter portion of the season the rains had been copious and well distributed, with low ranges in the desiccation and moderate and uniform temperatures. The comparison of the two censuses of this season with those of previous years shows different responses in the population to the conditions of

the total complex in which they were existing. Figures 58 and 59 illustrate well the condition in the population, and the fact that the observer who saw only the 1905 generations would have continuity, and the one that observed the populations of 1904 would have "mutations," and the stage would be set for a fine series of pointless "brief communications."



1655 Males. Total 3350. 1695 Females.

FIG. 58.—Census of first annual generation at Chapultepec colony in 1906, showing the array of population in pronotal pattern.



1842 Males. Total 3549. 1707 Females.

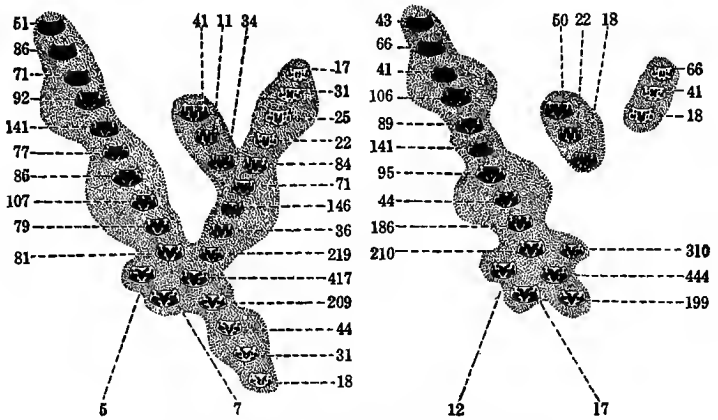
FIG. 59.—Census of second annual generation at Chapultepec colony in 1906, showing the array in the pronotal pattern.

The season of 1907 was in many respects an optimum one, with the different factors of the climatic complexes distributed in such fashion that they afforded the best conditions for the growth of the population at all times and essentially equal conditions in the early and late portions of the season, so that the conditions in the population were unusually uniform throughout the season.

The first census was made in the middle of July at the maximum of the first summer generation, and showed full development of all the lines, with the exception of biotypes 5 and 6, which were not well represented, if present at all. No isolated groups were found, with the exception of the females where

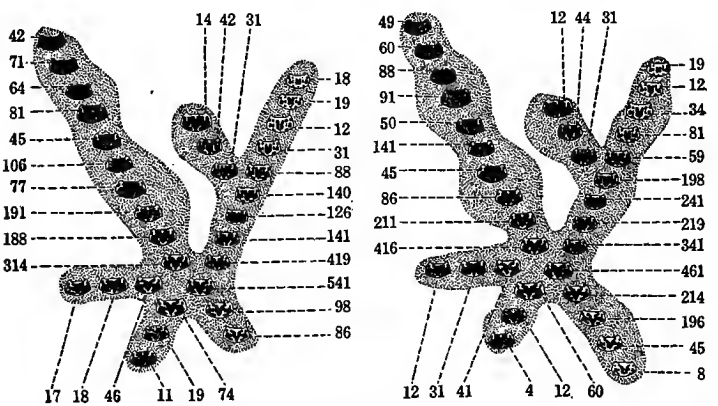
biotypes 10 and 11 were separated by slight gaps, and in the females the extreme end of biotype 8 was wanting.

The second generation in this season showed the nearly complete development of all of the lines in the population, no biotypic groups being absent, and none separated from the mass of the population by discontinuities of any sort. This



2289 Males. Total 4507. 2218 Females.

FIG. 60.—Census of first annual generation at Chapultepec colony in 1907, showing great difference in the array presented by the two censuses in the pronotal pattern and also different from those present in the preceding censuses.

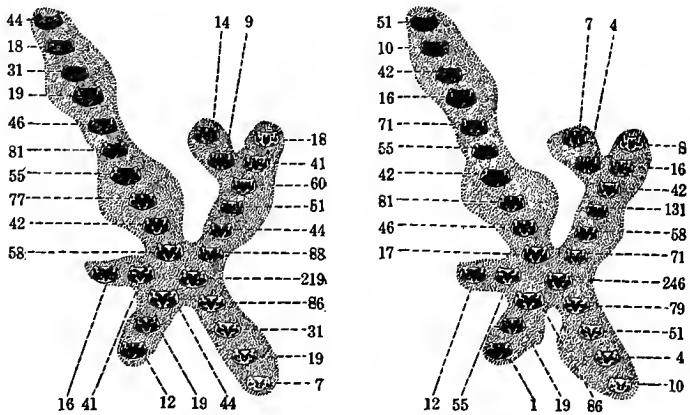


3170 Males. Total 6782. 3612 Females.

FIG. 61.—Census of second annual generation at Chapultepec colony in 1907. The population was numerous and showed nearly the complete array possible for the pattern in the population. Only a few of the extreme members in each of the general lines are absent.

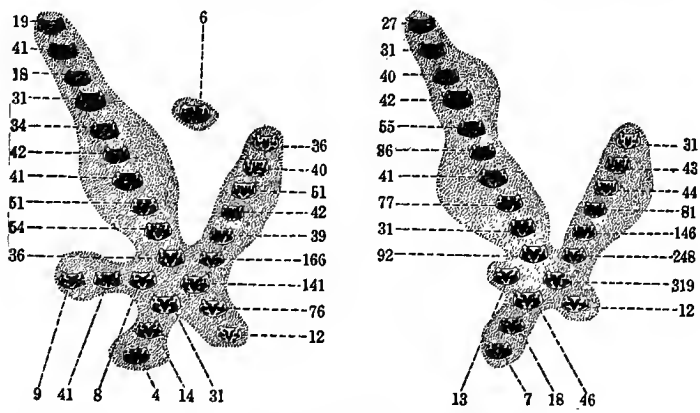
condition in the population is not often attained at any location in this species, and appears apparently only under the influence of optimum conditions in the climatic complex. The findings in the two censuses of the season of 1907 are shown in figures 60 and 61, the conditions of the climatic complex in climatic record 4.

The season of 1908 was also a highly favorable one for this species at this location, and therefore the results of the examinations of the population are much like those of the previous year. In the first census the population shows some curtailment of biotypes 5 and 6, the absence of 12, the reduction of 10, about equally in both sexes, so that the distribution of the sexes is essentially the same.



1290 Males. Total 2621. 1331 Females.

FIG. 62.—Census of first annual generation at Chapultepec colony in 1908, showing about the same range of pattern distribution, but with fewer numbers throughout and a tendency to restrict the extreme numbers, especially in biotypes 10, 11, and 12.

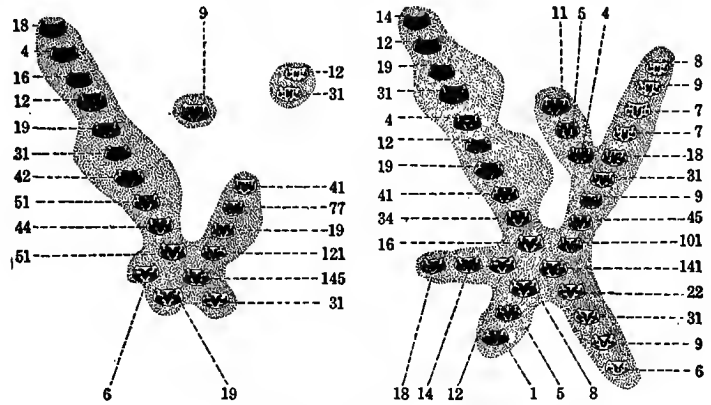


1083 Males. Total 2613. 1530 Females.

FIG. 63.—Census of second annual generation at Chapultepec colony in 1908, showing the array in the pronotal pattern of the population.

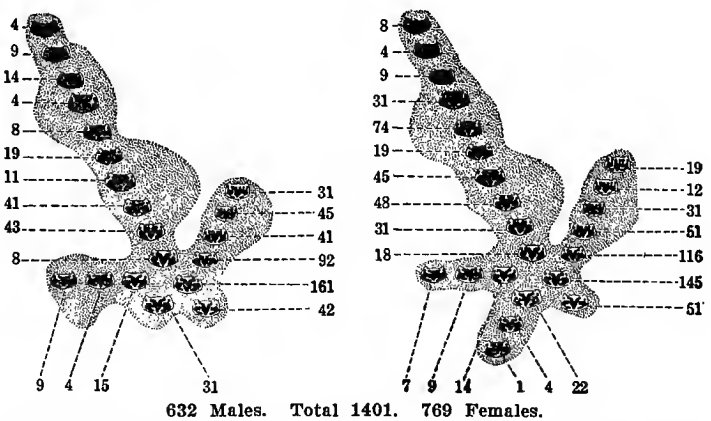
The second census taken in this season showed much the same conditions that were found in the first, with the exception that biotype 10 was present in the males as an isolated group and wanting in the female population. Some minor differences in the proportions of biotypes 5, 6, and 8 are shown in the array of the two sexes, but are not of any significance. The conditions of the habitat as shown by the meteorological records were much the same as in the previous year—uniformity of distribution of the elements and lack of extreme conditions. The findings for the two censuses are given in figures 62 and 63.

The season of 1909 was the last in which this series was followed methodically in this location, and completed a series of 12 censuses of 12 successive generations at one location, in a population of fairly restricted character and habitat. The first census of this season did not show any conditions greatly different from those found in previous determinations. The season opened with rains at unusually early dates, so that there was much reproductive activity in late May,



799 Males. Total 1523. 724 Females.

FIG. 64.—Census of first annual generation at Chapultepec colony in 1909, showing the array in the pronotal pattern.



632 Males. Total 1401. 769 Females.

FIG. 65.—Census of second annual generation at Chapultepec colony in 1909, showing the array of the pronotal pattern.

with widely ranging conditions in the humidity, rainfall, and desiccation. The population that started its development in this period showed that the males were much reduced in all biotype lines, with the exception of 1, 2, 3, and 4, the absence of 12, and portions of 11, 10, and 9, while the extreme of 10 and part of 11 were present as isolated groups. The females, however, showed nearly all of the lines present to their full extent, and no isolated groups, so that the conditions in the two sexes in the population were quite different.

The second census was taken at the optimum of the season in the second annual generation, when the conditions were somewhat more severe than they had been in this portion of the season in previous years. In this census there were no isolated groups in either sex; biotypes 10, 11 and 12 were wanting in the population in both sexes; biotype 8 was reduced to the merest trace, and might have been absent entirely; while biotypes 5 and 6 were variable in their presence in the sexes, but were certainly present in both. The general condition in the population in this year shows a uniform restriction in the range which might in some measure be connected up with the prevailingly harder conditions of the environment in the location during this season. The findings in the distribution of the population are given in figures 64 and 65.

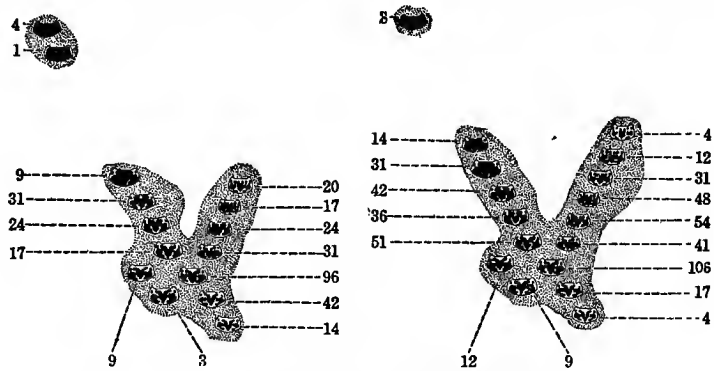
These 12 censuses, taken in the same location, when each of the two annual generations were at their maximum, do not show for the location uniformity in the population as a mass, but diversity. During the progress of the observations the climatic conditions were given as full study and measurement as possible, and while it would have been vastly better to have had measurements made on the spot, by methods better suited to the purposes of the study, the government observations are the best on the whole that are available for the entire period of the observations, and do show in rough fashion the march of events in the habitat.

The population is constantly shifting in this location, and has shown in the distribution of the quality chosen for examination—conditions which if seen but once would have been quite differently interpreted. It would in this instance not be difficult to build up from the data of this set of observations rather plausible arguments concerning the action of the environmental conditions in the production of the diversified arrays presented in the different censuses. I have never been enthusiastic in this, because all that I have been able to perceive in the situation is that the determination in nature has given me data concerning the sequence of events in two series of phenomena that were passing at the same place and time, and in some instances the oscillations in the one coincided in some respects with the oscillations in the other, but in so far as I am able to discover, there exists in these determinations plausibilities and suggestive situations that may well be the motive for exact experimental analysis in nature and in the laboratory. It is situations of this sort that have given the basis for numerous arguments in faunistic and ecological literature, as well as in the field of evolution essayism, in which the argument is from effect to cause.

THE TEXCOCO COLONY.

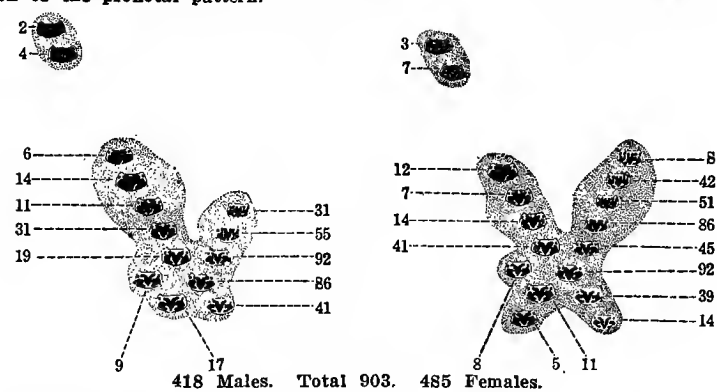
The records at this colony cover the period from 1903 to the middle of 1909, 13 censuses in the population being made, of which the first two were from collections made in the summer of 1903. The location and its general topographic setting have been previously described and differ from the Chapultepec location in many respects. For a record of the climatic conditions prevailing at this location I have been indebted to the local observers connected with the service in the state of Mexico. These are not so complete as those from the observatory at Tacubaya, and in the main are monthly averages, with distribution of some of the elements. These records, while more might be desired, are far better than none at all and are the best that one can do in work of this sort where trained observers are not available to make the proper measurements.

The first conditions in this colony were determined from collections made at random in the year 1903. The first census for this location shows at once a decided difference from the condition prevailing in the Chapultepec colony. Conspicuously isolated from the mass of the population is a group composed of biotype 1, while 2 and 3 are wanting in this line of development in both the sexes. The biotypes 10, 11, and 12 are also wanting in both sexes, and both show 9 fairly well developed, while 5, 6, and 8 are poorly developed, if represented at all. The distribution in the population is uniform for the sexes.



342 Males. Total 857. 515 Females.

FIG. 66.—Census of first annual generation at Texcoco in 1903, showing condition of the pronotal pattern.



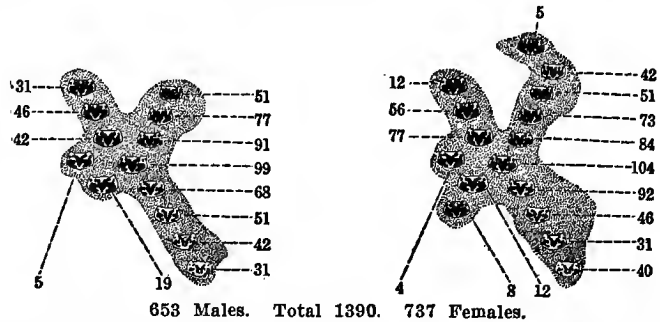
418 Males. Total 903. 485 Females.

FIG. 67.—Census of second annual generation at Texcoco in 1903, showing condition of the pronotal pattern.

The second generation from this location of which the census was made showed essentially the same condition in the population as was found in the first yearly generation. These determinations are shown in figures 66 and 67.

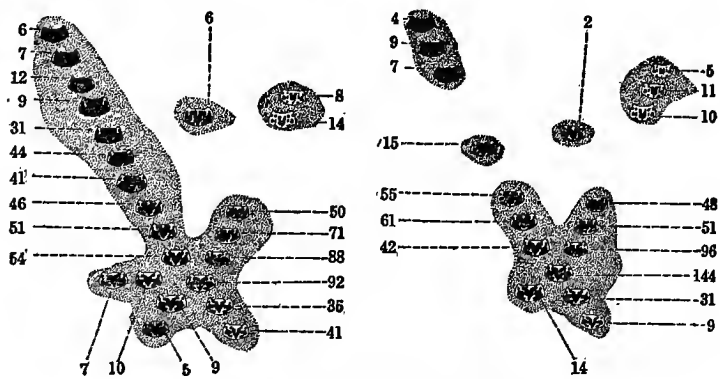
In the season of 1904 two censuses were taken; the first showing in both sexes considerable restriction of the range in the character, with the complete absence of any isolated groups and no trace of the isolated biotype 1 group that was found in the previous year. Biotypes 1, 2, and 3 were entirely wanting, as were 10, 11, and 12 in the males and 11 and 12 in the females. In both biotypes 5 and 6 were wanting or weak, but in both 8 was well developed in the population. Compared with the corresponding generation of the Chapultepec colony this population is strikingly different.

In the second generation of this year the distribution of the population over the range of values was much different from that of the first. The line composed of biotypes 1, 2, 3, and 4 was nearly restored in the population, completely so in the males and with only the lack of 2 and 3 in the females, while in both sexes 10 was present and isolated, as was also 11, while 5, 6, and 8 were wanting or weak in their manifestation. The conditions at this location seemed in this year to be much more variable than at the Chapultepec colony, and this seemed in some respects to be reflected in the behavior of the population. The findings in the censuses are shown in figures 68 and 69.



653 Males. Total 1390. 737 Females.

FIG. 68.—Census of first annual generation at Texcoco in 1904, showing condition of the pronotal pattern.



738 Males. Total 1352. 614 Females.

FIG. 69.—Census of second annual generation at Texcoco in 1904, showing condition in the pronotal pattern and increased variation thereof, but with marked gaps between the extreme members.

In the season of 1905 the population of the colony showed even more diversity in the range and in the number of isolated groups than in the previous season. In the first census made this year, biotype 1 was present as a conspicuously isolated group, not numerous, but vigorous in appearance, and would easily have passed in the population as a distinct species. In the males, biotypes 12, 11, 10, and 9 were all present and formed a continuous series, but in the females 11 was wanting, leaving 10 and 12 separated in groups. Biotype 8 was practically wanting in both sexes and 6 was absent from the female population, and represented in the males by a small isolated group. Biotype 5 was present in both, but in the males it was broken, the two extremes existing, with the average conditions wanting, while the females presented a uniform array in this series.

The second generation in this season showed still different conditions from that of the first, in that there was an entire absence of biotypes 1, 2, and 3, in both sexes; 10 was represented by a well-defined group that while small in numbers stood well apart from the population, and the female showed a weak development of 12, while 8 was wanting in both. Biotypes 5 and 6 showed the same general conditions that were found in the first generation of this year. Comparison of the findings in this generation of the population with that in the

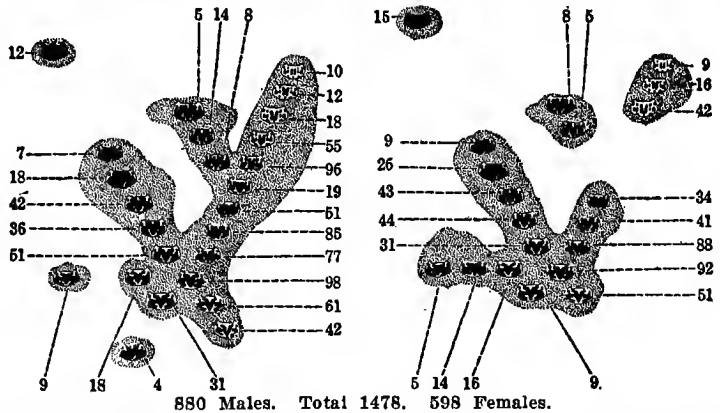


FIG. 70.—Census of first annual generation at Texcoco in 1905, showing condition of the population with regard to the pronotal pattern and in this isolated group.

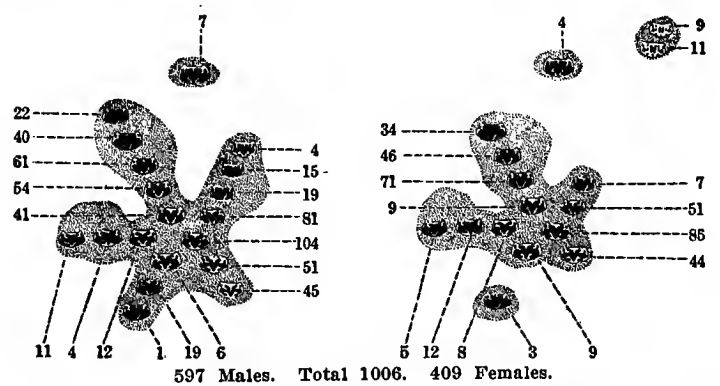
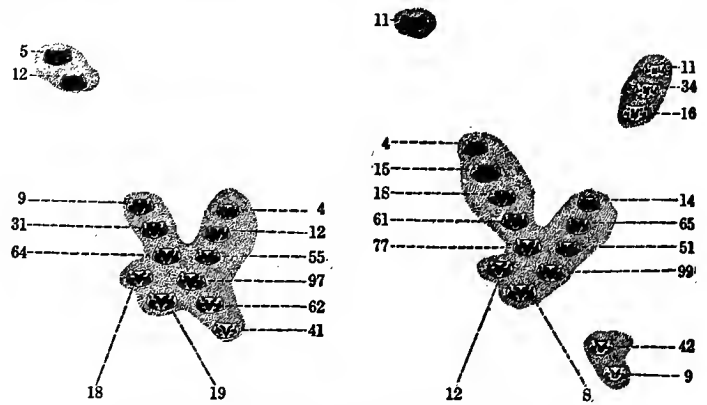


FIG. 71.—Census of second annual generation at Texcoco in 1905, showing reduction in the array presented by the population and the diminished number in isolated groups.

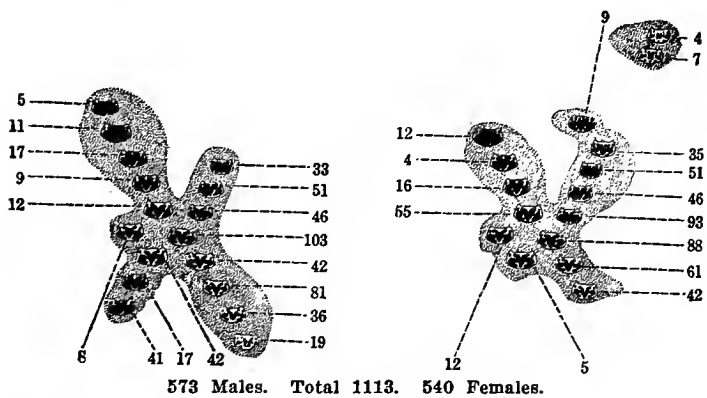
corresponding generation at Chapultepec still shows differences that are of considerable magnitude, chief of which is the evident tendency in the Texcoco location not to present so wide a range in the character in question, or the frequent complete series in the different lines, and the greater frequency of groups isolated by considerable gaps. The conditions of life on the whole were more severe at the Texcoco location, with wider ranges in the temperature and desiccation and more variable rainfall. Coupled with these conditions is the apparent difference in the population shown in the records. The findings for the two censuses of 1905 are shown in figures 70 and 71.

In the season of 1906 the first generation showed no especial change from the conditions shown at the close of the previous year. In both sexes biotype 5 was much reduced, 6 was also reduced, and 8 represented by a few at the upper end in the males and by a distinctly isolated group in the females. Biotype 1 was present in both in small numbers, and 11 was found only in the females. The two distributions are essentially the same.



429 Males. Total 976. 547 Females.

FIG. 72.—Census of first annual generation at Texcoco in 1906, showing continuation of the same condition present in the last generation of previous year.

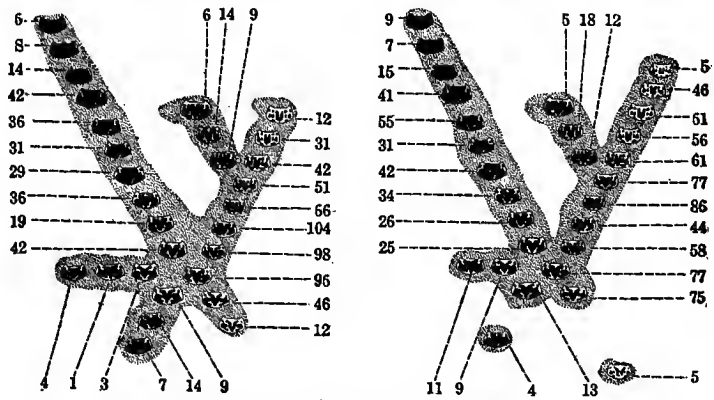


573 Males. Total 1113. 540 Females.

FIG. 73.—Census of second annual generation at Texcoco in 1906, showing condition with regard to pronotal pattern.

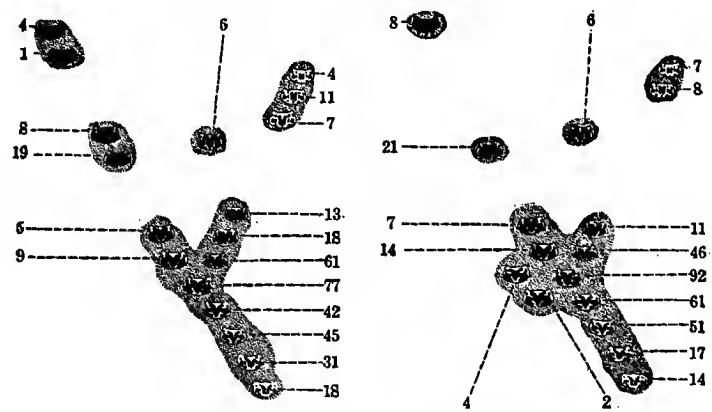
In the census of the second generation, biotype 1 was absent in both sexes and 5 was represented by a mere trace; 6 and 8 were fairly shown in the males, but in the females were weakly represented, while 9, 10, and 12 were best developed in the females, where 12 was found as a distinctly isolated group, the only one in the entire population. The records of the two generations are shown in the figures 72 and 73. Comparison of these two generations in the season of 1906 at Texcoco and at Chapultepec shows differences that seem permanent to the two locations in the population living there. In this year, at the Chapultepec

colony, there was a wide range in the character of the population with no well-developed isolated groups, but in the Texcoco colony the sharp restrictions of the central mass of the population and the evident propensity to produce widely and sharply isolated groups would seem to indicate for the two a different condition either in the population, in the medium, or both. Comparison of the conditions in the two locations on the basis of the climatic data that I can give is not satisfactory. In the latter part of the season of 1906 the conditions as regards



876 Males. Total 1874. 998 Females.

FIG. 74.—Census of first annual generation at Texcoco in 1907, showing sudden marked extension of pronotal pattern in the array in all directions.



378 Males. Total 747. 369 Females.

FIG. 75.—Census of second annual generation at Texcoco in 1907, showing reduction of the general mass of the population as far as pronotal pattern is concerned and increased number of isolated groups.

the precipitation and its distribution were more uniform and propitious to the development of this species than common, a fact that is partly indicated on the climatic record by the high rainfall in the latter portion of the season, which was also well distributed. The somewhat lower temperatures in this portion of the season acted perhaps to check or counteract the opportunity presented by the precipitation for the population to show wider ranges than common, which it did not do.

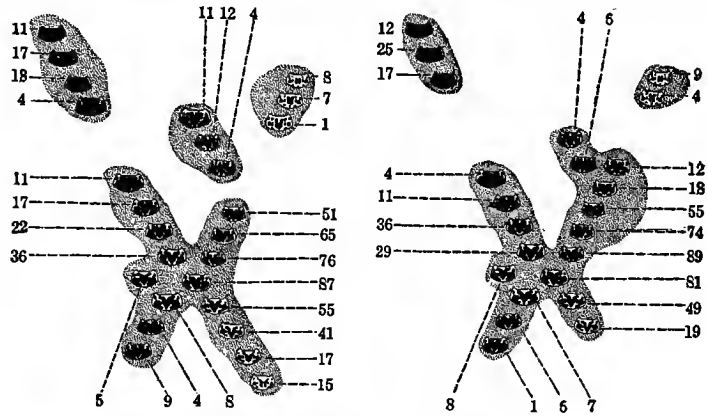
In the season of 1907 this favorable condition existing in the population at the close of 1906, and the still more favorable conditions in the early portion of 1907, with plentiful, well-distributed precipitation and the higher temperatures of late May and June, were the series of conditions that preceded and ensued during the production of the first generation in this year, and which showed the unusual condition for this location of almost no isolated groups and widely ranging distribution of the character over the possible range of manifestations. In both sexes biotypes 1, 2, 3, 4, 5, 7, 9, 10, 11, and 12 were present in full intensity, while 6 and 8 were weakly present in the males and in the females as the only two isolated groups in the entire population. The condition of the population in this generation is for once not unlike that in the Chapultepec colony in corresponding generation.

The second generation in this season showed the rapidity with which the population may change its aspect on the advent of an efficient cause. In this generation the solid, widely distributed condition of the pattern was again disturbed, and in its stead there was a narrowly restricted central mass of the population, and several sharply isolated groups. In the population biotypes 2, 4, 5, 6, and 11 were entirely wanting in both sexes; 9 was present as a weak development in both sexes; 8 was strongly marked in both males and females. Biotypes 1, 3, 10, and 12 showed distinctly segregated groups of considerable numbers in each instance, so that the aspect of the population at this time presented an entirely different one from that shown in the first generation of this season. The record of the two censuses are shown in figures 74 and 75.

The season of 1908 was favorable at this location on the whole, and from the point of view of climatic conditions there should have been a condition not unlike that in the Chapultepec colony at the same time. The census of the first generation for this season showed the characteristic, much-restricted central mass of the population and three large groups of isolated patterns. In the census biotypes 1 and 2 as a large group, 12 and 10 in the males, and 12 in the females were sharply separated from the rest of the population. Within the mass of the population biotypes 6 and 8 showed strong development in both sexes. The second generation at the time of census-taking showed essentially the same state as in the first generation, with the entire absence of biotype 12, and some minor changes in the rest of the population. The combined group, consisting of biotypes 1 and 2, was still strong and prominent in the population, in marked contrast to the condition of these groups in some other generations that had been examined earlier in the series. The results of the censuses for this season I have given in figures 76 and 77.

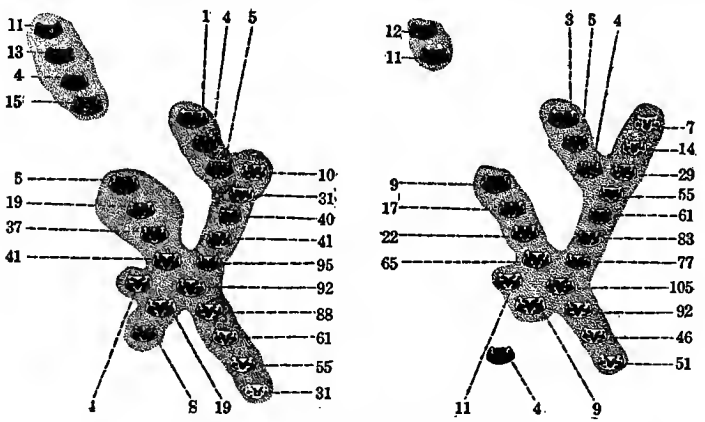
In the following year (1909) the colony was visited once in the early portion of the season, and the last census of the population made in the series at Texcoco. The condition in the population then found showed as in the first generation of 1907 that the conditions or something in the complex, either within or without the organisms, had produced an array of pattern conditions that was on the whole the widest that had been found in this location during the observations, and on the whole it presented a uniform distribution over the range of the possible arrangements. No marked or large groups isolated from the population were found and the three shown in the males are unimportant and few in number. The findings in this census are shown in figure 78.

Compared with the Chapultepec population for corresponding generations, this series at the Texcoco colony show constant differences, chief of which is the uniform restriction of the mass of the population to a rather narrow range in and about biotype 7; and second, the striking presence in the population of distinctly isolated groups, none of which have any constancy of numbers or occurrence. With the onset of environmental conditions which at other locations are opti-



612 Males. Total 1186. 574 Females.

FIG. 76.—Census of first annual generation at Texcoco in 1908, showing presence of numerous isolated groups of considerable strength and an increased array in the general population.

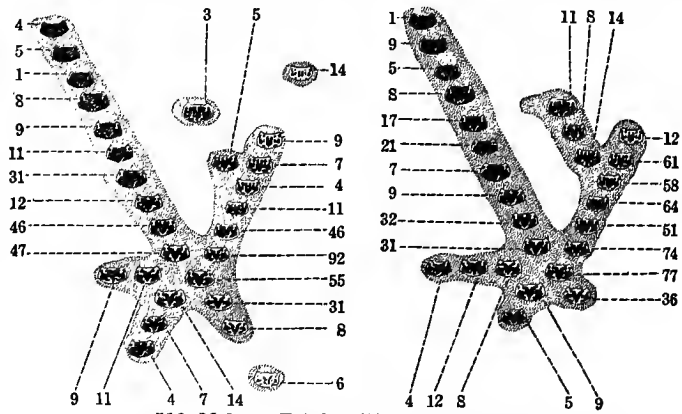


730 Males. Total 1522. 792 Females.

FIG. 77.—Census of second annual generation at Texcoco in 1908, showing condition in pronotal pattern.

mum conditions in the medium, the population at Texcoco was on the whole inclined to exhibit an increased response to the conditions in the form of widened ranges of the pattern combinations presented, which may be cause and effect, but not necessarily so. Commonly it would be so considered and the relation considered as established; but it is in any such "establishment" assumed that the material is uniform at the two locations, which may or may not be true. If the populations were uniform, then the certainty of such determinations

would be increased, but the populations are rarely uniform in two separate locations, and this is especially so in the case of organisms that are restricted to narrow habitats, and from which they do not move far. In such restricted habitats small peculiarities may and often do develop and become the potent cause of wide differences in the reaction of two supposedly like organisms to the same set of conditions in the environment. There is one outcome of these determinations of the conditions in these populations that is of interest and of some moment as a practical consideration in investigations of this nature, namely, the



510 Males. Total 1154. 644 Females.

FIG. 78.—Census of first annual generation at Texcoco in 1909, showing condition of pronotal pattern and extension of pattern in several directions, so a fairly continuous whole is presented with only small isolated groups in the males.

difficulty of finding twice the same condition in the state or array of the population in successive generations or seasons. The significance of this will appear more evident later.

THE TLALNEPANTLA COLONY.

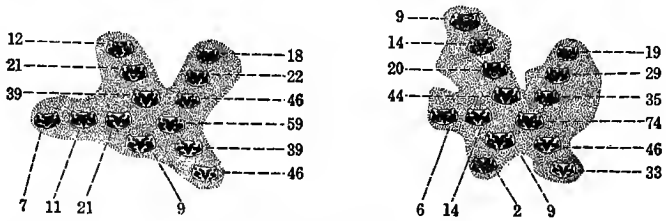
This location, in the northern portion of the valley of Mexico, the last of the locations in that physiographic unit, has been described, and it presents a different physiographic setting and, owing to local topography and climatic conditions, the same in plan and range, but differing in intensity and distribution in the growing-season of the year. On the whole, the conditions at this location are more severe than at either of the other two, and this in general finds corresponding changes in the response of the population of our subject living there.

The first census recorded from that location is from a collection made in the second generation of 1903; the remainder, to the end of 1907, have been made without removal of any of the population from the location. This was especially necessary in this location during most of the period of observation, owing to the relatively small number of inhabitants of this particular kind found in the location.

The condition found in the second generation of the season of 1903 indicated a narrowly limited range in the population without isolated groups in the population. This was true for both sexes. Biotypes 1, 2, 3, 10, 11, and 12 were entirely lacking in the population, and 6 and 8 were but poorly developed

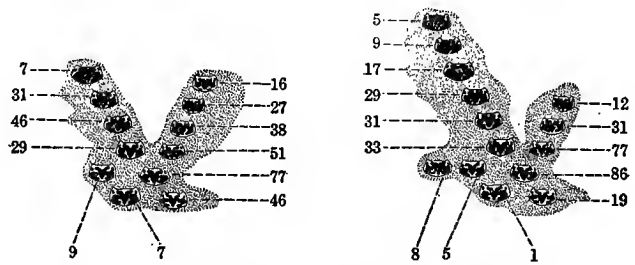
in the array, which might have been due to the small number in the original collection in this generation. The findings in this generation are given in figure 79.

In the season of 1904 two censuses were made, comparable in time with those at the two other colonies in the valley of Mexico. The first one for the first generation did not exhibit any considerable change from the condition presented in the population of the preceding year, with the exception of the better develop-



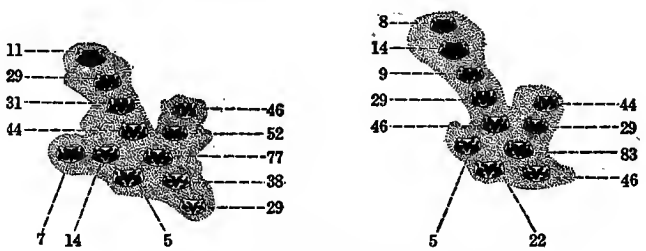
350 Males. Total 704. 354 Females.

FIG. 79.—Census of second annual generation at Tlalnepantla in 1903, showing conditions of pronotal pattern.



384 Males. Total 747. 363 Females.

FIG. 80.—Census of first annual generation at Tlalnepantla in 1904, showing condition of the pronotal pattern at that time.



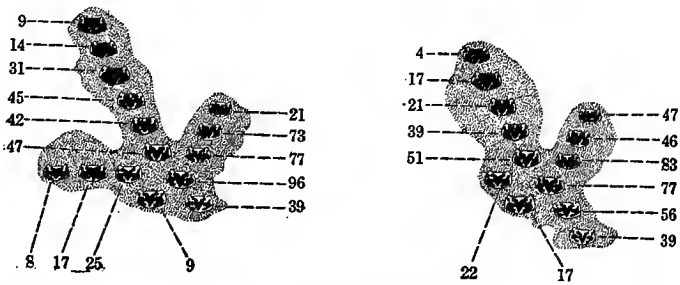
383 Males. Total 718. 335 Females.

FIG. 81.—Census of second annual generation at Tlalnepantla in 1904, showing the presence of a single isolated group of females, the rest of the population fairly uniform in its distribution.

ment of biotype 4 and a few biotype 3 individuals in the female population. On the whole, the population was massed on or near to biotype 7, the pivotal one in the series. The second generation did not show anything different from the first—only a somewhat more restricted range in the extremes of the population. The conditions as found are recorded in figures 80 and 81. In amount and dis-

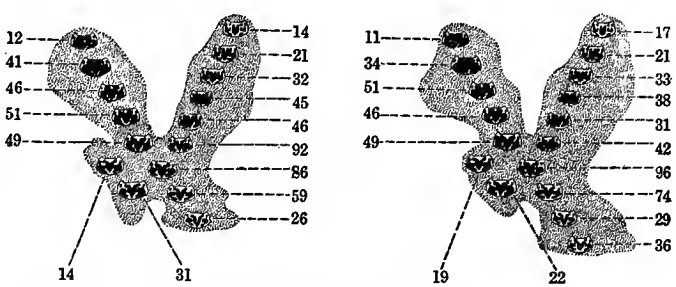
tribution of precipitation, temperature, and other factors the climatic complex was such as to indicate optimum conditions, basing the estimate of the conditions upon the conditions that had been found to be optimum in other locations. In the Tlalnepantla habitat, however, no response to the conditions was manifested in the population, such as had been observed in other locations.

In 1905 the first generation in the location showed some change, but no isolated groups, the area of the distribution of the pattern being "continuous," but showing development along the direction of the biotypes 3 and 4, also in the direction of 9 and 11. Biotypes 5, 6, and 8 were feebly represented in both sexes. In the second generation of this year the census showed the same con-



553 Males. Total 1072. 519 Females.

FIG. 82.—Census of first annual generation at Tlalnepantla in 1905, showing condition of the pronotal pattern.



665 Males. Total 1314. 649 Females.

FIG. 83.—Census of second annual generation at Tlalnepantla in 1905, showing an increased array in pronotal pattern.

ditions as in the previous generations. None of the isolated groups that were so characteristic of other locations was found, and the poor development of the more divergent biotypes in the population is characteristic of the location. Biotypes 3 and 4 were fairly well developed, as was 5 in the males, otherwise the census showed nothing of interest. The record of the censuses of these two generations are shown in figures 82 and 83.

The year 1906 showed in the location the first indication of any of the divergent groups, there appearing in the males biotype 1, which were found in freshly emerging condition, showing that they were a portion of the population, but

whether they were the product of an introduced individual of this biotype or not is impossible to determine. The remainder of the population showed only the customary array at this location in both sexes. The second generation in this year showed biotype 1 in both males and females as widely isolated groups. The remainder of the population was fairly well massed, but showed indications of extending the range of pattern in several directions. The recorded conditions I have also shown in figures 84 and 85.

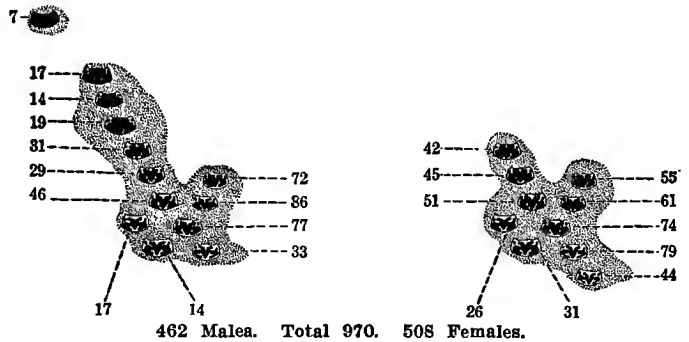


FIG. 84.—Census of first annual generation at Tlalnepantla in 1906, showing the array in pronotal pattern. In this generation there appears the introduction (apparently) of biotype 1 in the pronotal pattern which was present only in the males.

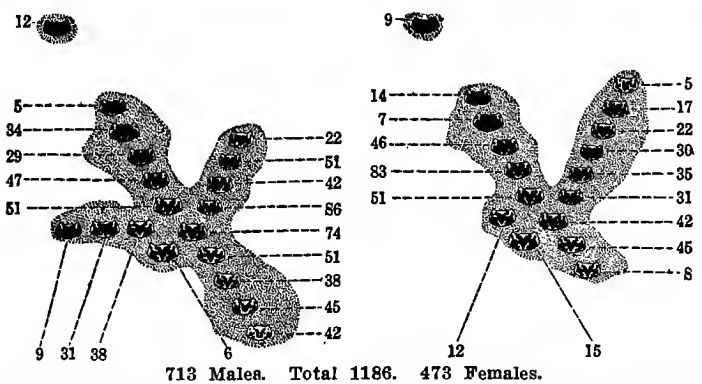
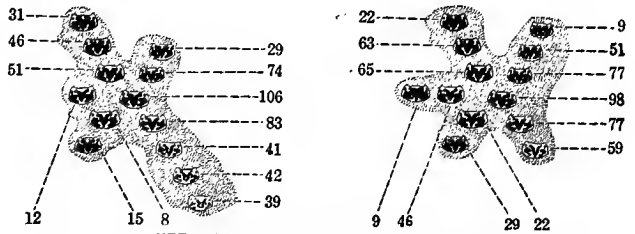


FIG. 85.—Census of second annual generation at Tlalnepantla in 1906, showing the presence of biotype 1 in both sexes and an increased array present in the general mass of population.

In the year 1907, the last at this location, the first generation showed no indications of isolated groups and only a much restricted range in the pattern, nearly identical to that found in previous years. The census of the second generation showed the usual well-defined and restricted mass, with little tendency to diverge, and isolated groups of biotypes 1, 8, 10, and 12 in the males, and of 1 and 8 in the females, all of which were fairly strong in numbers and

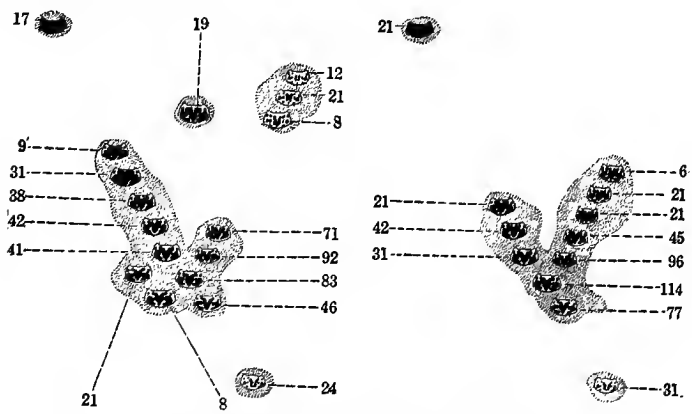
were clearly the product of the breeding of the population that had preceded, and not due to introductions. These records I have shown in figures 86 and 87.

The following year (1908), and again in 1909, the location was visited and a rough census made of the conditions in the population, but with no added indication of change in the characters of the population of the place. The same isolated groups occurred as in the previous years, and with frequency and in such numbers that it was evident that their occurrence in the location was not entirely the product of introductions, but was, in the main, the result of the response of the population to the conditions of the habitat into which they were by chance placed.



577 Males. Total 1204. 627 Females.

FIG. 86.—Census of first annual generation at Tlalnepantla in 1907, showing the pattern conditions.



583 Males. Total 1109. 526 Females.

FIG. 87.—Census of second annual generation at Tlalnepantla in 1907, showing the restriction of the mass of the population to a narrow range and the existence of several distinctly separate groups in both sexes.

The series of observation at the Tlalnepantla colony were in the character of the population, as indicated by these censuses, quite different from those in the other locations examined. Here as elsewhere the attempt to tie the observed conditions to any portion of or to the environmental conditions as a whole is argument from effect to cause or possible cause. The series may and often does make a plausible array, one that strongly indicates the relation of cause and effect.

Comparison of the conditions presented in the population at the three locations in the valley of Mexico shows decided differences in the character of the individual generations and in the sum total at each of the colonies. The Chapultepec colony presents the most diversity and the greatest range. Tex-

coco is next in the series, and the Tlalnepantla location least diverse in its population. The diversity is indicated in another way by the occurrence of biotype 1, which may with certainty be expected in the population of the Chapultepec colony. At Texcoco it is about a 1 to 1 chance that one will find it in the population, while at Tlalnepantla, the chances are far less, on the average 10 to 1, against finding it present. With reference to the other biotypes much the same set of chances might be derived, differing of course in the chances and in the locations.

That these three locations, situated on the rim of this great basin, presented constant differences in the character of the population was at first surprising, and when was added to this the weight of the determination from other locations in the basin, giving still other differences in the character of the population, so that at no two points in the whole area was I able to find like conditions in the population of this species, it became increasingly clear that the phenomena were well worth analysis. There has been little trace of migration of the population from one colony to another, although this has been sought for most diligently in the years of the study of the locations. Especially in the close of the season, in the interim between the maturing of the second summer generation and its entrance into hibernation for the dry season, there was opportunity for dispersion of the population at any colony to nearby locations, producing thereby a mixing and keeping the population of the valley fairly uniform. Any movements would, on account of the topography, be almost entirely confined to the valley of Mexico, and the only probable chance of foreign individuals would be from the plains of Apam entering through the pass at the northeast corner of the valley. All other sides were well cut off by topographic or other barriers that in the main prevented either entrance or exit to this basin. One exception to this must be noted in that, in especially favorable seasons, when the country to the north of the basin has had high temperatures and plenty of rain, there might be produced opportunity for food and dissemination, such that there might be entrance or exit on the north. However, I have not been able in two seasons of good conditions in these northern districts to discover any movement in either directions, so that I have come to regard the basin as one that is practically physiographically isolated as far as this form is concerned, as it is known to be for other organisms, especially those of aquatic habitat (Meek). It seems from all that I have been able to find out about the dissemination of these forms in the valley with respect to the regions about, that there is relatively little interchange as a regular thing, and only in exceptional years is there passage of many of the barriers to dissemination.

Within the valley there is of course more or less in the way of interchange of materials between the nearby locations where the animals are able to grow, although this is not as extensive as might at first seem probable. In the northern species, *L. decemlineata,* it has long been known that the autumn is a time of extensive dissemination by means of flying, and this is influenced by the winds to a considerable degree as has been shown in an earlier publication. In this species, in which the flying habit in the end of the growing season is also developed, I was interested to find if it there too played any considerable part in the dissemination of the animals from one location into another. Observation in the field established the fact that the forms did fly more readily at this season than at any other, and in captivity they do the same, so that the behavior is not a

product of the external conditions, or at least not entirely so, but in nature I have not been able to find them flying for more than a few yards at any one time. Thus far I have never seen one of these beetles fly more than 75 yards at any one time in nature, and after such a flight I have never seen it repeated, or rarely do they fly again until after some time has elapsed, and on resuming flight they are as apt to return to the original place as to start out in a forward direction, so that the end-result of the flying would, as far as I have seen it, be relatively little or no dissemination at all. From witnessing single exceptional flight and assuming that all flights were in a forward direction, quite false conclusions would be reached. I have followed a single animal for hours at a time in the proper season, with net result of the day's movements—10 or 12 yards' movement from the original starting-point and still within the original colony. On one occasion I followed one female three days at the Chapultepec location. The first day was passed in aimless walking of undetermined length, 87 meters of flight, and 5 hours of rest on the underside of leaves, passing the night in the ground in a crack beside a stone. This was at the height of the flying season in 1905. I returned to the location in the early morning and found the beetle in the same location, and as the morning was cool it did not move from the location until 10:23 o'clock, spent the time until noon walking and feeding on the nearby plants, took one short flight of less than 10 meters, and then came to rest on the top of a plant in almost the identical spot from which it had started the day before, moved about on the plant for a few moments, and then crawled down the plant to its foot and under some leaves at the base, from which it did not emerge. I left the location at 5 o'clock in the evening, returning the next morning at sunrise, and found the animal in the position in which it was left the night before. About mid-day it crawled up on the plant, remaining about an hour, but the dry, desiccating winds of the early afternoon soon drove it down again into the loose leaves at the foot of the plant, where at 2 o'clock in the afternoon it began burrowing its way into the soil, and in a half hour had passed out of sight.

This I have every reason to believe is a typical behavior of these animals in these locations; at least nothing to the contrary has been seen to indicate differently. It seems probable that in these restricted locations there are relatively few instances of interchange in the population, and especially so in those chosen for the examinations made.

In the course of this study a considerable number of locations of a highly restricted character was investigated, and while most of these were too small to provide the numbers that it was felt should be examined for the work, they did indicate a remarkable degree of difference as a result of the isolation which the topography and the climatic conditions enforced. For example, several locations in the region of the huge larva-flow in the neighborhood of Tlalpam showed exactly this condition in the character of the population, and some of these I saw at least yearly for 6 or more years.

This condition, prevailing in the valley of Mexico, received further confirmation from the data derived in the examination of the populations at the Puebla station and at the location near Chalcicomula, which were so isolated and separated from the three locations already examined that any interchange of population is highly improbable, or at least is not of common enough occurrence to be of any moment in analyses as crude as we are, at present, able to make in nature

with the methods of the census-taker. Both locations present differing climatic complexes, different topographic conditions, and, as might be expected, presented different aspects in the character of the population. *A priori,* it might be expected that the populations would differ in the two locations. The problem is, how and why do they differ? The "how different" is of no importance, excepting as it helps in the solution of the method of becoming different and the analysis of this operation.

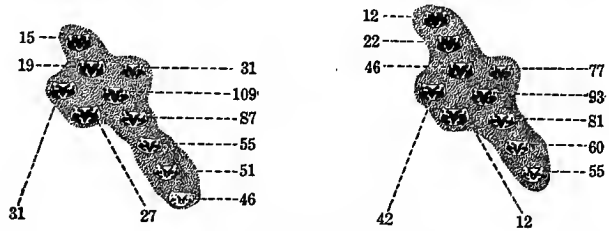
THE CHALCICOMULA COLONY.

This location on the western slope of the volcano of Citlaltepetl differs in many respects from the location in the valley of Mexico and the colony at Puebla. Situated in the lee of the huge volcanic pile, at an altitude of 8,500 feet, it was, as a result of the physiographic conditions, a dry, cold, grass-covered plain, with a short growing-season and cold nights, even in the best of seasons. In this general region the development of the colonies of this form were not at any time on a par with those at Puebla or at Chapultepec in the valley of Mexico. The location chosen for examination was 2 miles west and north of the town of Chalcicomula and about 1 mile west of the Barrio de San Augustin, in the open plain, with rainy-season pools. Observations were begun in 1904 and continued until the middle of 1910, covering 13 generations in the location. The censuses at this location were not as satisfactory as those at other locations, owing to the small numbers and the frequent difficulty of being sure that a considerable number of the previous population had not persisted and formed a part of those present at the second census in the season. This was due to the frequent spell of low temperature in the summer months, which kept the individuals alive, inactive, and present in the population longer than in other locations. This error exists in any census determinations and can hardly be compensated for or eliminated unless the individual by being killed is put beyond the possibility of again entering into the count. The determinations, defective as they may be, show decided differences between this colony and those previously studied.

In the first year of observation (1904), the first census showed a small population, which was centered around biotype 7 in both sexes and with 8 well developed in both. No divergent or isolated groups were found in the population. The records are shown in figures 88 and 89. The second census, made in September, showed the same general condition, a small population, aggregated closely about biotype 7, and in this generation 8 was not represented any more than the others that center immediately about the pivotal biotype. Comparison of these two censuses with those made in the same year at the other locations shows a quite different condition than exists elsewhere.

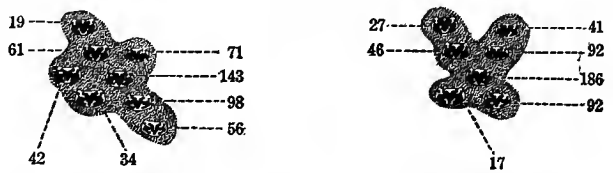
In the season of 1905 the first census made showed in the males a strong development of biotype 8 and something of 4 and 5, but in the female the condition was not changed. No isolated groups of any kind were found in the location. The second determination for the year showed in both sexes about the same condition that existed in the males of the previous generation, this time without divergent or isolated groups. The records of the conditions found are shown in figures 90 and 91. The two generations of this year compare well in range and numbers with those of the previous years, the four showing that the location is more constant in the appearance of the population than any of the other locations examined.

In 1906 two generations were again examined, with the results shown in figures 92 and 93, no essential change being found in the population, the only item of interest being the existence of the extreme of biotype 8 present in the



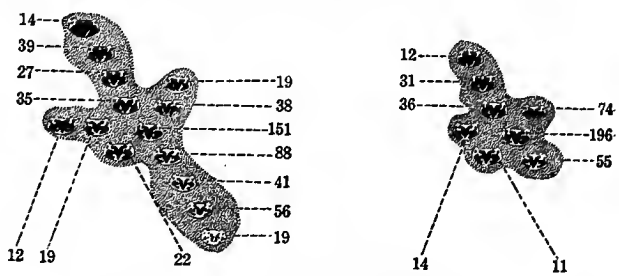
471 Males. Total 971. 500 Females.

FIG. 88.—Census of first annual generation at Chalcicomula in 1904, showing condition in pronotal pattern, and the restriction thereof to a very narrow range.



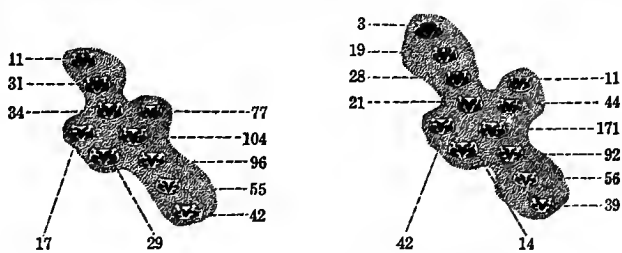
524 Males. Total 1025. 501 Females.

FIG. 89.—Census of second annual generation at Chalcicomula in 1904, showing further reduction in the range of pronotal pattern.



580 Males. Total 1009. 429 Females.

FIG. 90.—Census of first annual generation at Chalcicomula in 1905, showing a somewhat increased array of the pattern.

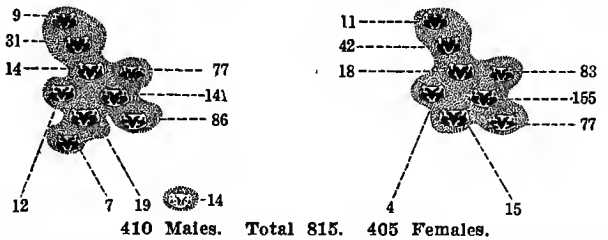


496 Males. Total 1036. 540 Females.

FIG. 91.—Census of second annual generation at Chalcicomula in 1905, showing the stable condition of the population with reference to pronotal pattern.

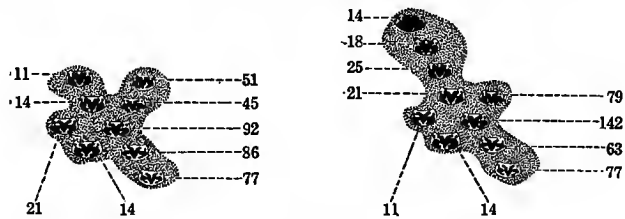
males as an isolated group. The population was small, and it is probable that twice as many would have shown intergrades in this direction.

Two censuses were taken in the season of 1907, with the results shown in figures 94 and 95, where no difference in the character of the population was found in either generation. There were now records of 8 generations without



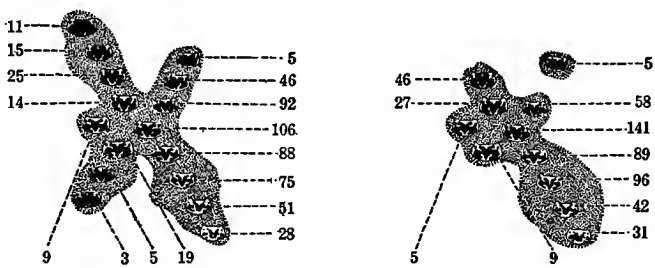
410 Males. Total 815. 405 Females.

FIG. 92.—Census of first annual generation at Chalcicomula in 1906, showing a reduction of the array in population.



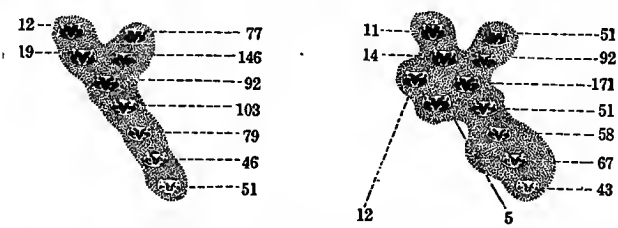
411 Males. Total 875. 464 Females.

FIG. 93.—Census of second annual generation at Chalcicomula in 1906, showing a marked reduction in the array.



592 Males. Total 1141. 549 Females.

FIG. 94.—Census of first annual generation at Chalcicomula in 1907, showing a somewhat increased array of conditions in the pronotal pattern.



625 Males. Total 1200. 575 Females.

FIG. 95.—Census of second annual generation at Chalcicomula in 1907, showing a marked reduction of the conditions in all lines with the exception of biotypes 8 and 9, into which the majority of the population seem to have been thrown.

trace of any of the behaviors found in the other locations, so at the end of this year a large sample was taken to Chicago for testing in the laboratory, and there were introduced into the colony in September 425 individuals at random from

the Chapultepec colony, which went into hibernation with the native population and passed the cold, dry season in the new location. The purpose of the introduction was to discover, if possible, whether the condition of the population as shown in the censuses was the result of the conditions of the environment or the constitution of the materials, which in some unknown manner had become limited to the narrow range of heterogeneity shown. It might be expected from the result of the introduction that the condition of the population would change in its nature, or that the new introduced conditions would be obliterated.

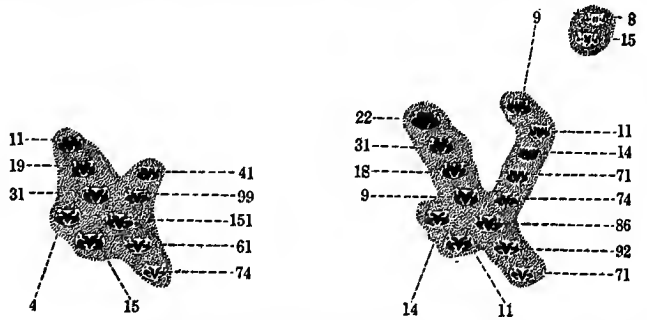


FIG. 96.—Census of first annual generation at Chalcicomula in 1908, showing return of the population to the condition observed most frequently in the location.

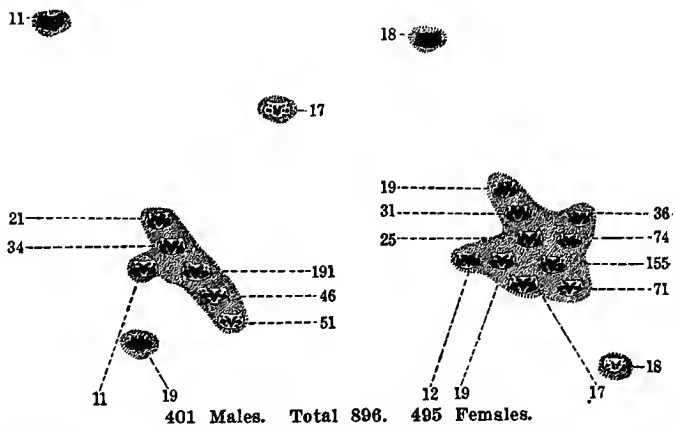
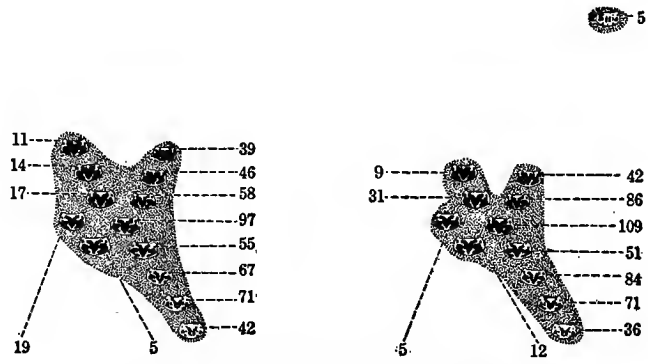


FIG. 97.—Census of second annual generation at Chalcicomula in 1908, showing presence of isolated groups of biotypes 1, 10, 9 and 7, after the introduction of the heterozygous mass from the outside. The population in general is closely massed around biotype 7 as in previous instances.

I do not know how many survived the cold, dry winter in this location, but when I saw the product of the native population and the introduced materials in the first summer generation in 1908 there was little trace of the introduced materials, with the exception of an isolated group of females of biotype 12, as is shown in the record in figure 96. The second census of the season showed the same uniform population, but present in it were divergent isolated groups of biotype 1 in both sexes, 6 and 12 in the males, and extreme 8 in the females. These groups were strong in proportion to the population (fig. 97).

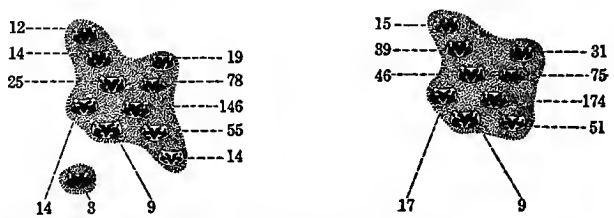
In the following season (1909), none of the introduced conditions were found in the population in either of the censuses, the only trace being a single deformed weak female of biotype 12 in the first generation of the season, which did not reappear in the second annual population. In the second generation a small group of isolated males of biotype 6 were present, but were too few in proportion to the total population to have any meaning (figs. 98, 99).

Evidently something that has happened in the location and in the population has obliterated the introduced stock in a relatively short time and held the state of the population at a fairly constant average, in spite of the disturbances of



541 Males. Total 1082. 541 Females.

FIG. 98.—Census of first annual generation at Chalcicomula in 1909, showing massing of population in or close to biotypes 7 and 8, with only a few present in the divergent group of biotype 11 in the females.



389 Males. Total 846. 457 Females.

FIG. 99.—Census of second annual generation at Chalcicomula in 1909, showing massing of population in still more restricted zones around biotype 7, practically eliminating all biotypes introduced from outside.

the introduced strains carrying divergent factorial conditions that might easily disrupt the population of this restricted area. The experiment is suggestive, but from it one can not decide whether the resulting rapid incorporation of the introduced stock and its characters is due to the dominant character of the native material, to the conditions of the environment, which either eliminated the introduced forms, or has made them recessive in the population, with final eradication from the constitution of the race at that localized area.

At the end of this season I introduced from the Puebla colony a large army of immigrants, 1,845 females and 2,134 females, a horde outnumbering the native population several times. These hibernated with the natives, and apparently

there emerged in the spring a large number, and they interbred with the native population and *inter se,* giving the resultant distribution of the population in the first generation shown in figure 100. In this array the mass of the population is still gathered about the same condition as in the other generations examined, and the development of the line composed of biotypes 1, 2, 3, and 4 is, relative to the entire population, weak and may well have been due to the inbreeding of some of the Puebla immigrants, of which all of these conditions were present in the population introduced, and not to the inbreeding of the native, and introduced with the dominance of the introduced over the native. Census methods could not decide this in nature.

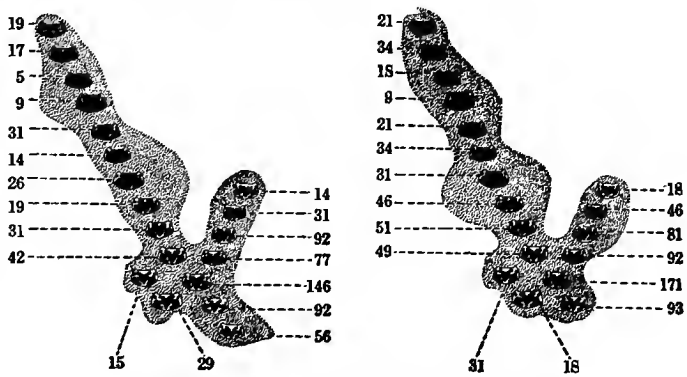


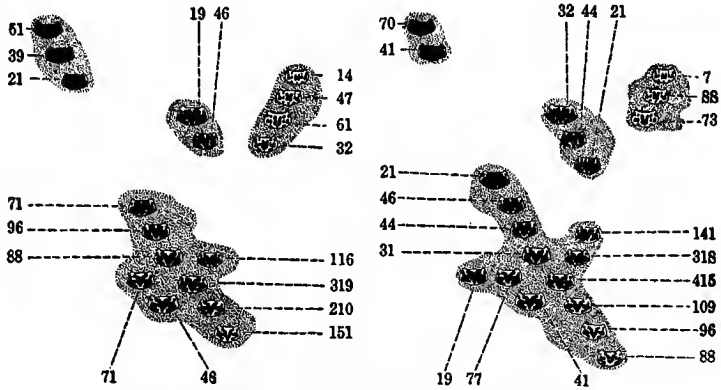
FIG. 100.—Census of first annual generation at Chalcicomula in 1910, showing array in population after the introduction of a large foreign population from Puebla.

I have no further records of the fate of the colony with its immigrants, owing to the disturbed condition of the area in the seasons of 1911 and 1912. An attempt to gain information of the condition of the colony in the winter of 1911-12 by digging for the hibernating beetles also gave no results, owing to the reluctance of the labor in town to go on a strange errand into the country. To judge by past experiences in the location, it is probable that the introduced material was progressively incorporated into the population, leaving its presence indicated for longer or shorter periods by changed ranges in the character most considered.

THE PUEBLA COLONY.

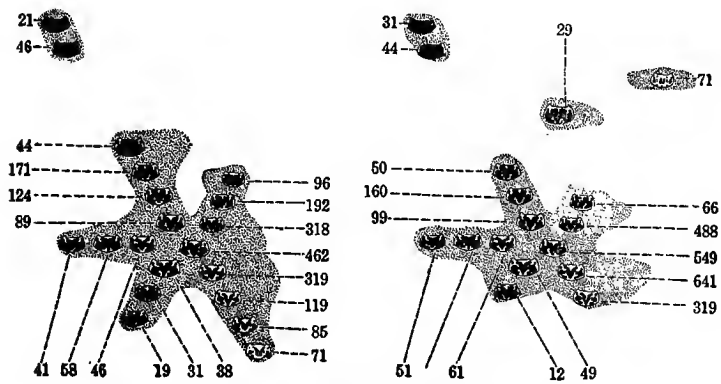
The location of this colony in the Rio Atoyac Valley, on the brink of the Mexican Plateau, in a location open to the warm winds from the low, hot valleys to the southward, and protected from the cold winds from the northern plateau and high volcanoes, with higher rainfall distributed over a somewhat longer portion of the year, gave the location a more favorable set of conditions than any of those previously examined, with the exception of Chapultepec, whose peculiar topographic situation gave it especial advantages over other locations in the valley of Mexico. At Puebla two collections near the location chosen, made in 1903, gave data showing the difference that existed in that year between this location and others examined, and in 1904 observations were begun and continued to the close of 1908, covering 10 generations in the location.

In the first census of 1904 the population showed a condition not unlike some of those already encountered in the first two examined, namely, a large central mass grouped about the central biotype 7, and isolated groups of biotypes 1, 10, and 12 in both sexes. In the second generation in this season the distribution of the population showed about the same condition of the central mass of the



1488 Males. Total 3310. 1822 Females.

FIG. 101.—Census of first annual generation at Puebla in 1904, showing condition of the pronotal pattern and presence of several strongly marked isolated groups.



2390 Males. Total 5010. 2620 Females.

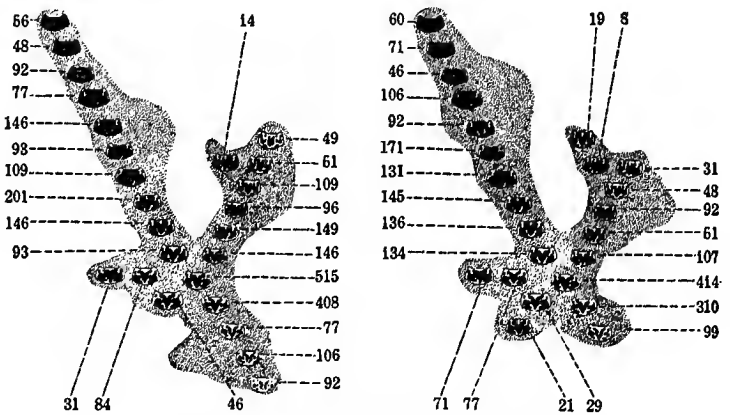
FIG. 102.—Census of second annual generation at Puebla in 1904, showing essentially the same conditions as found in previous censuses.

population and the same isolated groups, with the exception that in the males the groups composed of biotypes 10 and 12 were wanting. The records of the conditions in these two censuses are shown in figures 101 and 102.

In the season of 1905 both censuses showed the complete absence of isolated groups and a nearly complete distribution of the population over the entire area that the pattern covers. The season was especially favorable, with regularly

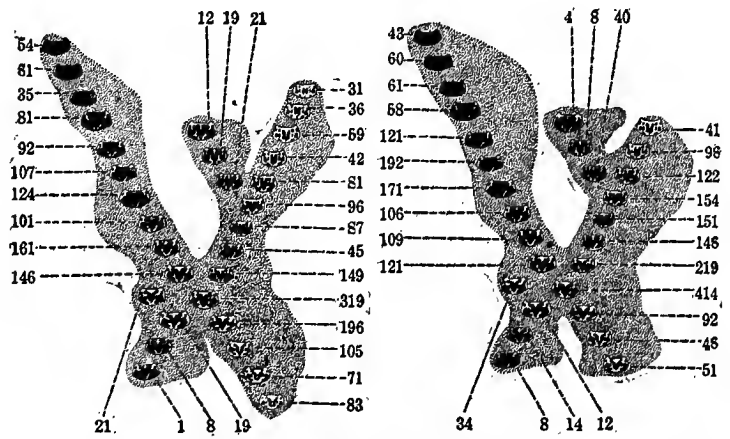
distributed precipitation and other conditions optimum in their arrangement. The findings in the population are shown in figures 103 and 104.

The year of 1906 was a favorable year as measured by the range in the character presented in the population, as shown in figure 105, wherein no separate



3034 Males. Total 5501. 2467 Females.

FIG. 103.—Census of first annual generation at Puebla in 1905, showing entire lack of isolated groups and relatively wide range of population with respect to conditions of pronotal pattern.

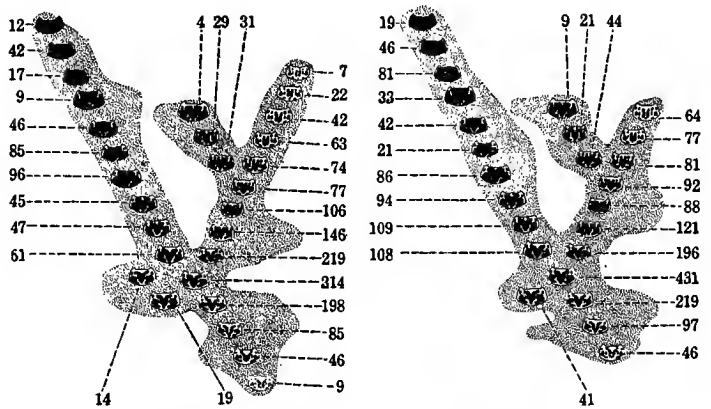


2463 Males. Total 5157. 2694 Females.

FIG. 104.—Census of second annual generation at Puebla in 1905, showing further increase in array of pattern and also a sharp increase in the population in regard to size. No isolated groups.

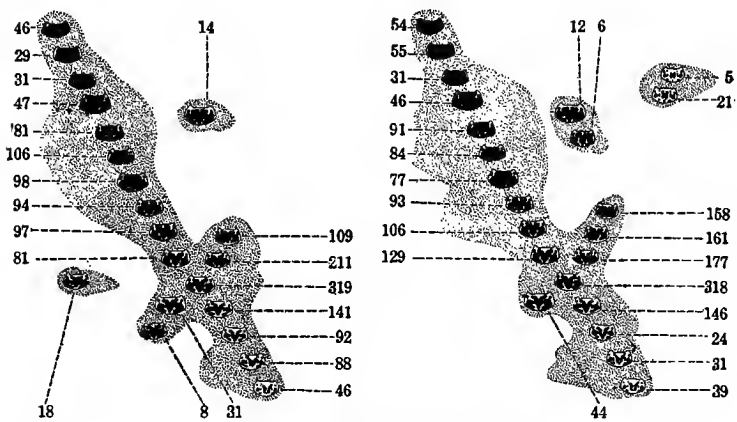
groups are present in the population; but the second generation of the year showed isolated groups again in biotypes 5 and 10 in the males and in 10 and 12 in the females, all relatively small in numbers, as shown in figure 106.

In the season of 1907 the first generation showed a curious reduction of the line composed of biotypes 1, 2, 3, and 4; 1 and 2 were entirely absent in the



1955 Males. Total 4221. 2266 Females.

FIG. 105.—Census of first annual generation at Puebla in 1906, showing same array of pattern conditions present in figure 104, but somewhat decreased in amount of fluctuating variability.



1787 Males. Total 3695. 1908 Females.

FIG. 106.—Census of second annual generation at Puebla in 1906, showing a restriction in the array of pattern conditions, the appearance of isolated groups and increased fluctuation in the line composed of biotypes 1, 2, 3, and 4.

males, while 1 was present and 2 and 3 absent in the females. The same condition prevailed in the second generation of this year, where biotype 1 was present in both sexes, but 2, 3, and most of 4 were absent, as were also 10, 11, and 12 in

both sexes. These conditions in the population are shown in figures 107 and 108. This behavior of the population in this season is difficult to account for. The season, as far as the recorded conditions are concerned, was not below those of the two preceding years, and on my visits to the location in the middle and end of the season nothing was discovered that would indicate that the climatic conditions had anything to do with the array presented by the population and the practical obliteration of the major portion of the biotypes in lines 1, 2, 3, and 4. I saw the second generation twice in its life—once during the time when it

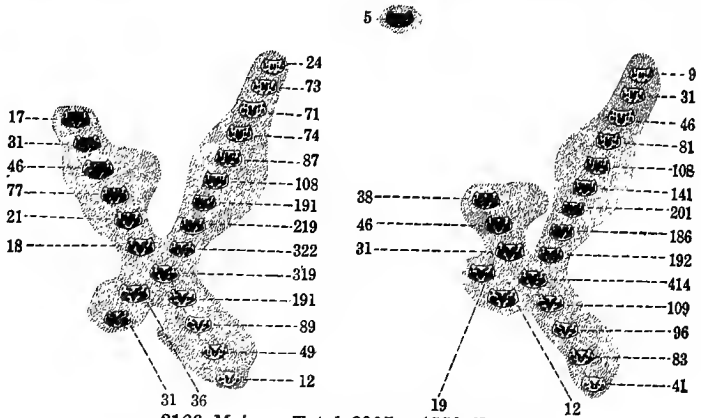


FIG. 107.—Census of first annual generation at Puebla in 1907, showing reduction in array in population and also absence of isolated groups.

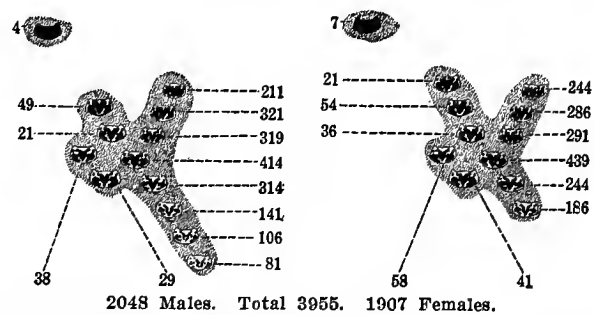


FIG. 108.—Census of second annual generation at Puebla in 1907, showing an extensive reduction, as far as the array is concerned, with isolated groups composed of biotypes 1 and 2 in both males and females.

was mainly in the larval and pupal stages and again when in the adult condition, and at no time was there any indication of an epidemic of disease or of parasitism which might have been responsible for the condition found. Further, I have never seen disease or parasitic attacks select out a portion of the population in this exact manner. The condition found is more probably the result of some condition in the population itself than entirely the product of external conditions, which may have been a contributing factor. Whatever the cause of the condition, it is all too evident that only experimental analysis of the situation and its experimental duplication can solve the problems involved. The census

and observation in nature can suggest possible solutions, but can solve none without the aid of analytical experiment.

In the following season (1908), the population was examined with much interest in the first generation to see if the condition prevailing in the previous season persisted into the following generations. The census of the first generation showed that the line of biotypes 1, 2, 3, and 4 were present in full in both sexes and strongly developed, as were 5, 6, and 8. In the 9, 10, 11, and 12 series

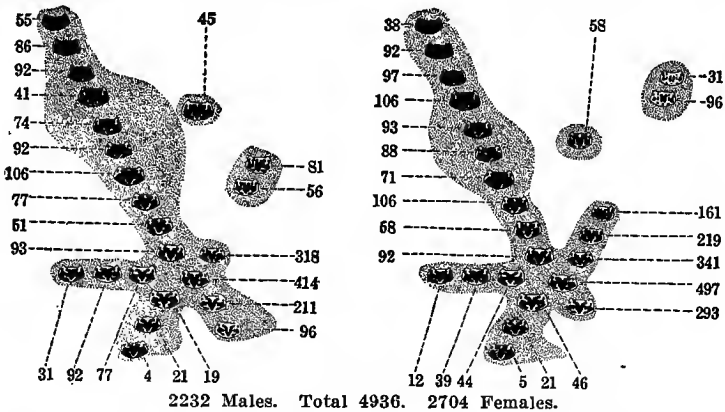


FIG. 109.—Census of first annual generation at Puebla in 1908, showing a sharp increase in the array in the population, with the presence of two added isolated groups in both males and females.

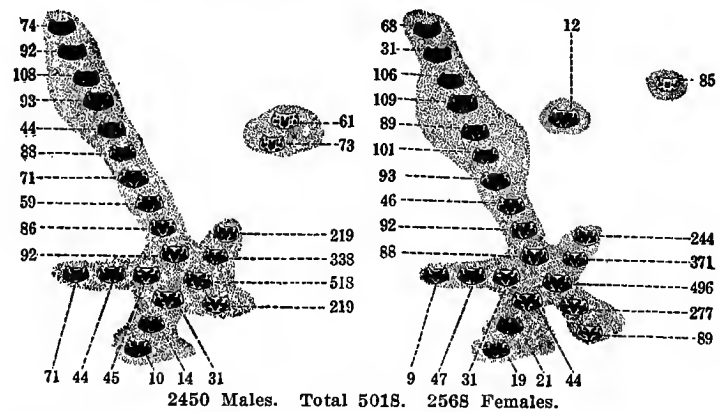


FIG. 110.—Census of second annual generation at Puebla in 1908, showing array and increased development along the line composed of biotypes 1, 2, 3, and 4, and isolated groups in 10, 11, and 12.

there was decided derangement, 10 being isolated in both, 11 in the males, and 12 in the females as distinct groups. In the second generation the same condition prevailed in the population, with the exception that 10 was absent in the males and only present in the females as a small group, while 11 was present in the males and 12 in the females. Otherwise the distribution of the population was uncommonly well developed. These conditions are shown in figures 109 and 110. In this season the conditions of the previous one were repeated, but

with a different series of the population involved in the operations. The climatic conditions in the location were, on the whole, as indicated in the records, more favorable for the entire population than in the previous season, both being of such a character that one would hardly look for extensive derangements in the array of the population due to the conditions of the environment. There is little doubt that the condition is a perfectly normal one in the population and that the change in its character in successive seasons and generations represents the product of operations that are far too delicate and intricate in their organization and operation to be analyzed by the crude methods that must at present be employed in investigations of this kind in nature. The fact that the climatic records are the product of a meteorological station of itself introduces a huge error, and they are not at this station, nor at any other station that might be chosen, of much service in determining the rôle of the external condition in the operations that take place in the organisms living there. I have not the least doubt that if it had been possible to have had proper observations at the location, some element in the complex of the environment would have been found that was playing a part in the production of the diversity observed.

COMPARISON OF THE FINDINGS IN THE COLONIES.

The conditions presented by the arrays observed in five locations, in different generations and seasons, show phenomena that are of interest and helpful in the further study of the problems of heterogeneity. Most striking is the manner in which this complex character, simple in comparison to the entire organism, shows a ceaseless movement over the field of possible form or pattern conditions in the part. In no two locations, at no two points in time, is one sure of finding the condition in the population the same, even in the array that is present, much less in the proportions and distribution of the population over the area it can occupy. The conditions found are fascinating and suggest vividly the manner in which some distribution enigmas may well have arisen. This production in the population of conditions, first in one direction, then in another, withdrawing from the first but dropping behind, isolated remnants, that conceivably might thenceforward become isolated permanently through diverse natural processes, and so a stem-form with the differing factorial potentialities that this one has might, without further endowment or other natural operations, be productive of a considerable amount of "species formation" as it moved over the substratum on which it lived. Figure 111 shows the conditions of all of the populations in outline, so that the nature of the movements become more striking in their diversity in the different locations and generations.

Another point of interest is the mass action of the population in its response from generation to generation and in the different locations, in the manifestation of the responses in "variation," either determinate or indeterminate, in the sense of Darwin. Both are in this series present together and manifested in the different locations in different ways. Each of the locations chosen showed that the populations respond differently to the complex in which they lived from the response shown in other locations at the same time and generation by the same "species." No question exists that in the Chalcicomula location the population responded definitely, uniformly, in the same manner, to the conditions of the medium, else why so certainly should the introduced strains have gone from

sight so soon? The Tlalnepantla colony also shows the same definite response in the population as a whole to the complex, and the Chapultepec and Puebla colonies each shows its characteristic difference in the population at the location. Thus each of the population masses varies in its location in differing degrees in several directions, and all may well be present at one time, producing a well-defined condition of "indefinite variation" in the population, which in turn may be followed by decided "definite variation" in Darwin's sense as the result of some minor change in the direction of incidence or intensity of some of the factors in the medium, of which instances are fairly frequent in the data presented.

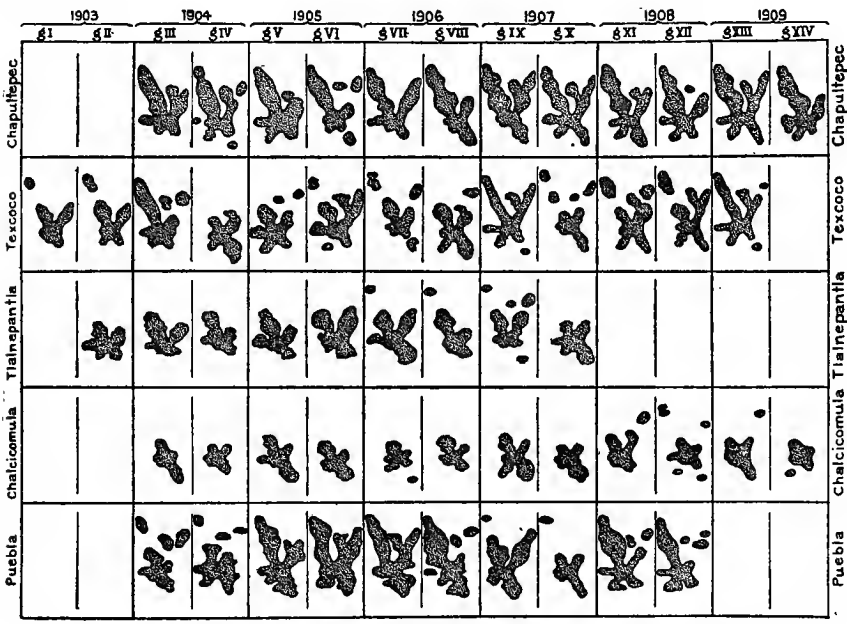


FIG. 111.—Composite figure representing results observed at five colonies described in preceding pages and change in the appearance of the population in successive generations through a period of years. Corresponding determinations are arranged one above the other, so that conditions prevailing in the population at a given time in the colonies are comparable. This figure shows graphically the behavior in the population which the different colonies presented and which are apparently constant conditions in the habitats studied.

The condition presented in a complex character of this kind, as a type of what one might expect to find in more complicated forms or in organisms as a whole were it possible to analyze the relations fully, is suggestive, and is one that may well have played a rôle in the production of the heterogeneity that exists in the organized world. Darwin certainly saw the conditions in nature in this respect and tried to estimate them on the basis of their value in species formation, on the basis of a working hypothesis that he was not able to put to an exhaustive experimental test. With Darwin, as since, it has been entirely a matter of opinion as to how conditions like these operated in nature to produce heterogeneity and stable permanent groups under natural conditions. This is still too much a matter of opinion and plausibilities, but should be a matter

of direct experimental analysis, in the laboratory and in nature, in at least a few instances. These materials do show precisely the condition that Darwin saw and considered in the variation phenomena in organisms and the condition upon which much of the controversy concerning variations, their direction and selection, have been based. In this an unfortunate cul-de-sac was produced by the biometricians following Galton, which for many years completely befogged the true situation as Darwin had seen it, and as it is now seen more clearly with the aid of present methods of analysis.

Throughout the series of observations there is constant delimited " variation," no trace of heterogeneous undelimited conditions being present, nor is such to be expected in a complex pattern-system of this sort, which is itself a product of the interactions of simpler elements, " simplest characters," each the product of an exact and demonstrable group of productive agents, so that the system dealt with, as far as it is known, is physically exact in its constitution and action, and delimited behavior is to be expected. Any other condition would be the basis for most searching analysis to discover hitherto unrecognized agents and to determine their rôle and place in the system.

This series of observations upon *L. multitæniata* Stål at the locations given has been supplemented and tested by the minor test series made at different points in the south Mexican region where this form is found. Some of these locations have been mentioned in an earlier portion of this chapter. The results at none of these were in any way different from those already described, and are not given, purely on account of the space they would occupy.

All that an examination of this sort can ever establish, even under the best of conditions in nature, with natural species, is the determination of the results of unknown antecedent series of causes. In the organic population a series of events is passing, certain stable conditions which are capable of convenient expression, in one form or another, and so in the medium another series is passing, some elements of which are measured, and the two are matched up as seems most plausible on the basis of some hypothesis which may or may not be true and applicable to the case in hand. The net result of an examination of materials such as is presented in the preceding pages is solely a determination of the range, extent, and direction of the diversity of the materials in nature, no more, and at the same time some of the conspicuous associations of the factors in the medium, and of their range and direction of diversity, and in its totality of both series, there is accomplished only the preliminary rough blocking-out of the problems that are necessary in any investigation. All further progress rests in this, as in all other instances, on the analysis of the materials and conditions, their relations, and the recombination of separated materials and elements by synthesis under divergent conditions, and in these laborious, complicated analytical and synthetic operations alone lies the sole basis of further knowledge.

One other point in this series of observations is of interest in this place, namely, the effect produced by the treatment of the data by the methods of the biometrician. If the series of data had been treated in this manner and only the amount of the pigment present been considered, the result of the examination would have been a series of polygons of distribution, in which the phenomena revealed by the analysis here employed would have been obliterated and the resultant curves meaningless. One could find different polygons at different

places and at the same place in different generations, which would then be interpreted on the basis of some favored hypothesis, and that would be the end of the study. The condition of the data thus gathered and analyzed left nothing upon which to initiate further investigations into the analysis of the "variation" phenomena that were present. The present method of analysis of the conditions in the character examined, and the same would be true of any other, does leave a broad foundation upon which to build further investigations.

Similar series of observations in other species in the group have been made and brief statements of the findings in some of these are presented, not that they add anything in principle to the conditions seen in *L. multitæniata,* but they show that the state found in that species is not limited to it, nor are the principles which it shows specific in any degree. This species is of the high-plateau group, and for comparison I shall give briefly the conditions in a lowland savannah species *L. undecimlineata* Stål and *L. decemlineata* of the temperate portions of the northern United States. Data of the former at two locations Tierra Blanca and San Marcos in the lowland savannahs of Mexico, at Chicago for the latter form in the north.

IN LEPTINOTARSA UNDECIMLINEATA.

The condition presented in the pattern of the pronotum of this species shows many points of likeness to the pattern in the species already examined. The same elements are present in the system, with some wanting, and a few are added. As in the former species, by proper breeding analyses it can be broken up into a series of groups that are uniform in character, breed true in mass cultures, and in all respects biotypic groups of this character and species. In figure 112 is shown the condition of the pattern as a whole, as far as it is known to exhibit differences in the pattern within the known range of the species in nature, representing the conditions over the whole known extent of the species. The known range shows that the different conditions can, for convenience, be arranged around the neutral or indifferent biotype 7. In *L. undecimlineata* the same elements are present as in *L. multitæniata,* the combinations in both are the same, and in both similar directions of the modification are found. From biotype 7 in both, 9, 8, 5, and 4 are present in the same condition, displaying the same elements in the identical combinations thereof, and with the same general range; biotype 6 is also present, but with an added element, the area forming a connecting element between *c* and *e,* and designated in this species as biotype 6*a*. Biotypes 1, 2, and 3 are uniformly absent in this species, and 4 is not as a rule much developed in any location thus

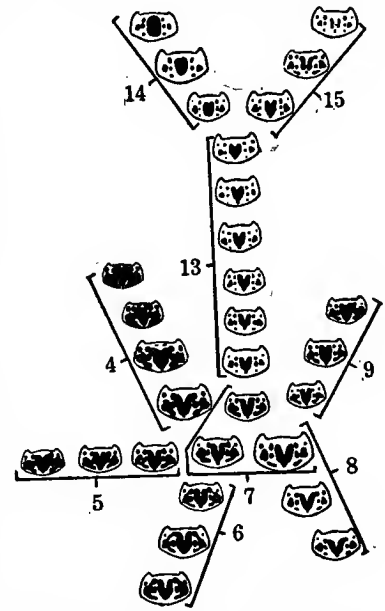


FIG. 112.—Diagram representing biotypes and their arrangement in the population in the species as *L. undecimlineata* and *L. panamensis.*

far seen. Biotypes 10, 11, and 12 are entirely absent, but a new line of arrangement is introduced in this form that is represented by biotypes 13, 14, and 15, pattern arrangements that were not present in the population of the first species.

ADDITIONAL PRIMARY BIOTYPES.

Biotype 13: A condition of the pattern in which the marginal elements are all wanting; the areas much reduced with the fusions $a'+a$ and $d+e$ the only ones present. There is also a reduction of the anterior *a* center and a widely divergent axis of the *a*, the spot giving them a sharp broad V-shape that is quite distinctive and unlike anything seen in *L. multitæniata*. Biotype 14 is in most respects like 13, with the exception that the *a* spots are parallel and fused along their entire median sides, giving a central oval or oval spot notched at the ends. This biotype is also distinctly separated from the others by the absence of spot *c*. Biotype 15 differs from all of the rest in the complete lack of any permanent associations between the elements of the pattern and the tendency to reduce the areas into their component elements where compound. The *a* areas are often separated, and the *e* areas are divided into a three-lobed area or into three isolated centers. Considerable somatic disturbance occurs in this pattern and often obscures the true condition in the system, as is shown in the figure, where the member of this biotype nearest to 13 and 14 shows the *a* areas fused, when in reality such an individual would not breed as such.

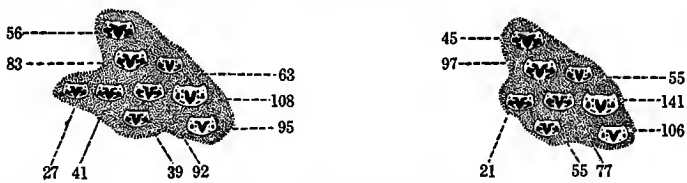
The two species then present 16 biotypic conditions that can be analyzed out of the populations, that are capable of being isolated, breed true, and act when crossed as alternatives, giving in F_2 a segregation into the same extracted biotypes. There is one conspicuous difference between the condition in *L. multitæniata* and *undecimlineata*, namely, that in the first the entire range in the character may be present in the population at one location, as it was at Chapultepec on certain occasions, while in *L. undecimlineata* the entire known range of the system is never exhibited by the population of any location at one time or through any length of time; and furthermore, the different biotypes have a geographical distribution in the habitats occupied by this species that is interesting and would have been of interest to have followed in a series of locations had this been practicable. The difficulties of transportation in the southern portions of the range inhabited by this species, from the Rio Coatzacoalcos and southward, made any project of this sort out of the question. Had such been possible, it is certain that the results of the examination would only have shown the same principles found in the locations that could be followed with ease, and have added perhaps instances of localized distribution of some biotypes, a condition that has little interest to us, and no possibility of solution. The fact that biotypes may be geographically limited is at least interesting and suggestive of another productive cause of heterogeneity in nature.

Two locations were chosen for examination of this species in the savannahs of lower Vera Cruz, on the Vera Cruz al Pacifico Railroad, that were comparable but different in topography and in the environmental complex, as far as it is possible in the savannah areas. These locations at Tierra Blanca and San Marcos have already been described in Chapter II in the description of the habitats and the sources of the material that had been used in the experimental work. No records of the climate for either place are available that are of any

value in this connection, and the few measurements and the maxima and minima that I obtained are not of any significance in this connection.

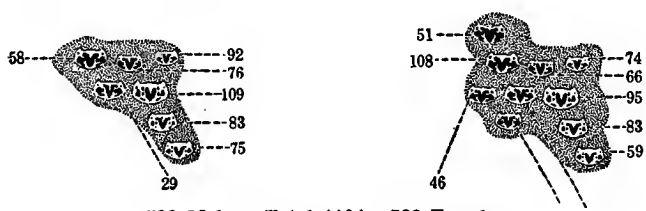
THE TIERRA BLANCA COLONY.

Eleven censuses of the population were made at this point, beginning in 1904 and ending in 1909. These probably do not represent as purely generation determinations as do those for *multitæniata,* owing to the fact that the species breeds over a longer time than the former, and that the generations become much mixed from the middle of the summer on, and in nature the population is mixed with respect to the number of generations in the yearly cycle. In captivity in pure lines the cycle in my cultures has been uniformly two, with a period of rest between that varies with the conditions of the medium and the stock. In nature the population is immensely diversified in this character, and stock from nature is heterozygous, giving all sorts of combinations at the start, so that the censuses taken here represent censuses of the natural population, in which the relations of generations to one another and to the censuses could not be determined with accuracy. This condition will not invalidate the general results that may come from the examination of the materials, and will give data on the species comparable with that already obtained.



604 Males. Total 1201. 597 Females.

FIG. 113.—First census of condition in the population at Tierra Blanca in 1904, showing the pronotal pattern.



522 Males. Total 1104. 582 Females.

FIG. 114.—Second census at Tierra Blanca in 1904.

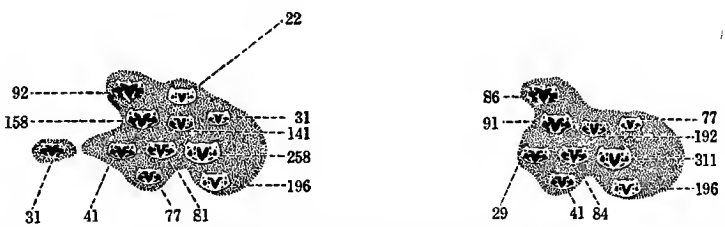
The first census of 1904 showed in both sexes a uniform population, centered mainly in biotype 7, with indications of 5, 6*a,* 8, and 9 poorly developed, and no isolated groups of any sort, and on the whole an extremely uniform set of individuals and homogeneous in the total mass of the population. The second census showed essentially the same condition in range and in the biotypes present, the chief difference being the increased development of 8. As far as I could observe and determine from the data to be obtained, the conditions of the climatic complex had been favorable to the colony in the year; at least no striking adverse conditions had been present. The records of the population at the two censuses are shown in figures 113 and 114.

In the season of 1905 the first census showed no change in the character of the population; if anything it was more completely limited to the central mass and almost entirely in biotype 7. The second determination showed increased ranges within the general mass of the population, and also the presence of marked, divergent groups that were well separated from the rest of the population. In the males there was a strong group of biotype 5, and in the females a strong one of 14 and a minor one of 15. The records of the two censuses are shown in figures 115 and 116. The environmental conditions, as far as known, showed no divergence of interest.

In the season of 1906, the first census showed an increase of the number and strength of the isolated groups and changes in the distribution of the population

1099 Males. Total 2300. 1201 Females.

FIG. 115.—First census at Tierra Blanca in 1905, showing restriction of the population to relatively narrow conditions centering around biotype 7.



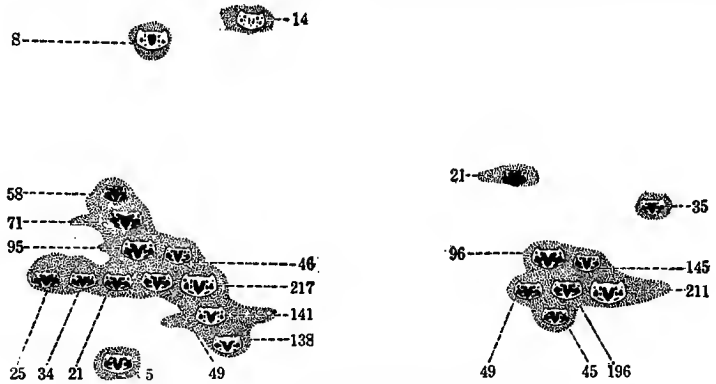
1128 Males. Total 2258. 1130 Females.

FIG. 116.—Second census at Tierra Blanca in 1905, showing somewhat increased array and the existence in the females of two highly divergent isolated groups.

as a whole, especially in the males. In this sex, biotypes 4 and 5 were strongly developed; 6*a* was present as a marked group isolated from the population, and 14 and 15 were also isolated and of considerable strength in the population. The females showed almost complete absence of biotypes, with the exception of 7, and an isolated group composed of the extremes of 4 and some few individuals of 9, both standing well apart from the mass of the population. In the second determination of the condition in the population a sharp change had taken place in that the mass of the population in range and in the groups that were present had been much extended in both sexes and was about equal in the array of biotypes presented. In both, 5, 6*a*, and 8 were strongly present; 4 was strong in the males but weak in the females; 7 strong in both; and in the males, 14 was present as a well-marked isolated group. The conditions found are given in figures 117 and 118. The only item to be noted in the environmental conditions

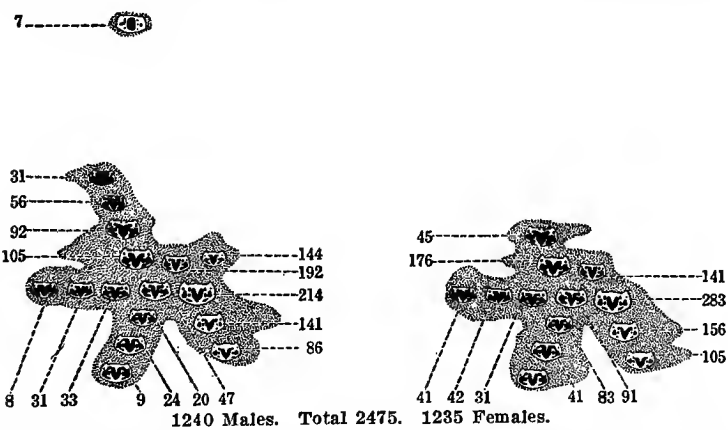
in this year is the early onset of the rainy season, the amount and uniform distribution of the precipitation, and accompanying this there was an uncommon development in growth and luxuriance of the flora and fauna noted throughout the season.

In 1907 the season was an average one, and in both determinations the population showed only restricted ranges in the immediate proximity of biotype 7,



922 Males. Total 1720. 798 Females.

FIG. 117.—First census at Tierra Blanca in 1906, showing condition in the population as a whole and existence of an increased number of isolated groups.



1240 Males. Total 2475. 1235 Females.

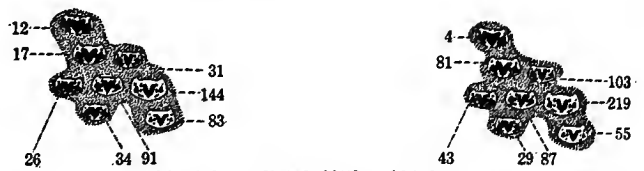
FIG. 118.—Second census at Tierra Blanca in 1906, showing increased array in the population as a whole and the restriction of isolated groups to a single one present in the males only.

with no divergent groups. The conditions of the population in the two censuses are shown in figures 119 and 120; distribution about like that in 1904 and 1905.

In 1908, which was a season in character like that of 1906, the population again showed increased ranges in the array of the central mass of the population and considerable developments of isolated groups. In the first census the two sexes showed essentially identical conditions; the central mass showed a heavy development of biotype 7 and conspicuous showing of 5. Biotype 4 was feebly

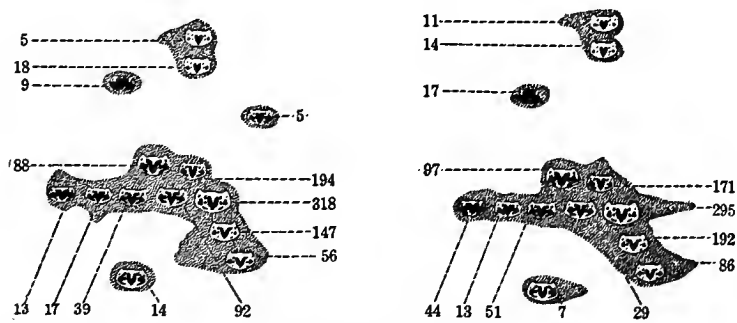
697 Males. Total 1535. 838 Females.

FIG. 119.—First census at Tierra Blanca in 1907 showing condition in the population with regard to pronotal pattern.



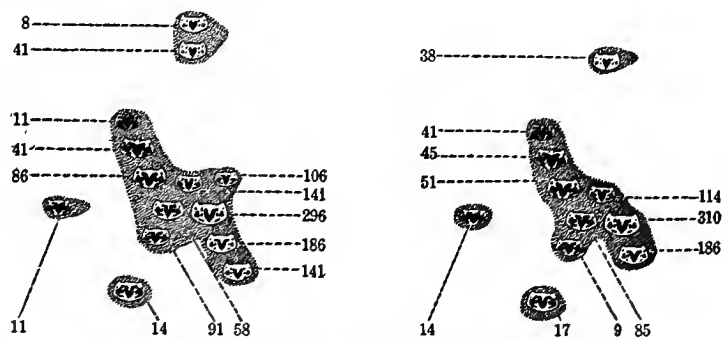
438 Males. Total 1059. 621 Females.

FIG. 120.—Second census at Tierra Blanca in 1907, showing condition in pronotal pattern.



946 Males. Total 1973. 1027 Females.

FIG. 121.—First census at Tierra Blanca in 1908, showing sudden increase in certain directions in pronotal pattern and the sudden rise of three strongly developed isolated biotypic groups in both sexes.



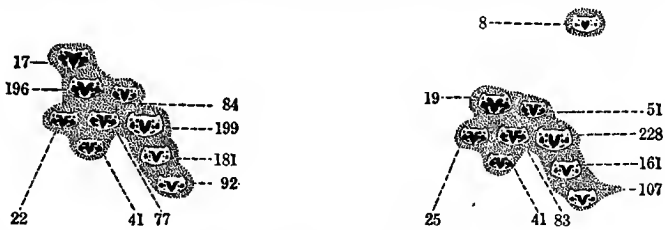
1231 Males. Total 2141. 910 Females.

FIG. 122.—Second census at Tierra Blanca in 1908, showing condition in the population as a whole and the persistence of isolated groups in previous censuses.

represented, and 8 was present in small numbers. In both sexes biotype 6*a* stood apart from the population as a marked group, as did the extreme conditions in 4 and a fairly strong group of 13. In the males some of the extreme conditions of 9 were present as a poorly developed group. In the second census the two sexes again showed the same conditions, in that the central mass had changed in its shape by cutting off the extremes of biotype 5 as an isolated group of some strength, and the increased development of 4 into a strong group. Biotypes 6*a* and 13 were still represented in the population by isolated groups. These conditions in the population are shown in figures 121 and 122.

The last census made at this location was the first in 1909 and showed little of interest. The body of the population showed the same condition that was seen in the last census of the previous season, but the isolated groups had dropped out, with the single exception of a small group of biotype 13 in the females (fig. 123).

In principle these arrays of the population through a series of generations and seasons does not show anything different from that found in the data of *L. multitæniata*. There are present in the population the same biotypes to some extent, showing that in part at least the same complex system is being dealt with, but in the population there appear other combinations that are not known in the



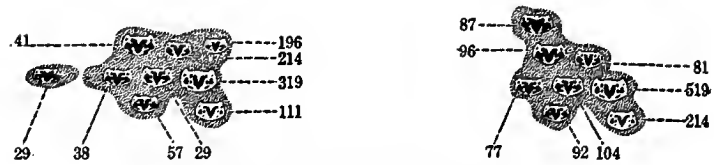
909 Males. Total 1632. 723 Females.

FIG. 123.—First census at Tierra Blanca in 1909, showing condition of the pronotal pattern.

former series, as in biotypes 13, 14, and 15. In this series the same ranging condition of the population over the possible pattern conditions is seen, but for the location the range is limited to a relatively narrow series of changes, and the isolated groups are uniformly conspicuously isolated from the mass of the population.

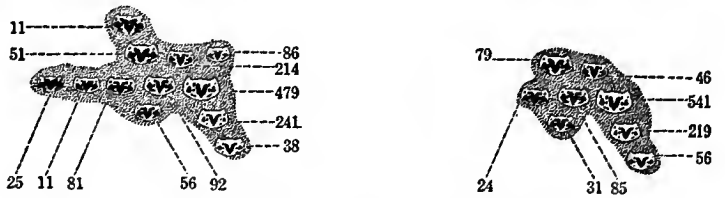
THE SAN MARCOS COLONY.

I introduce the data of this location briefly, as a comparison of two locations that are much alike in their topography, climate, and other conditions of life, covering the same time and the same number of determinations as in the first colony, but showing that the two are not at any time the same in population, even though the same elements may be present (figs. 124 to 134). Comparison of the two series of determinations given will show the differences found purely local in character, but of enough importance so that the differences might well be productive of unlike results in different investigations, unless the local differences in the constitution of the races are taken into consideration. For purposes of some of the latter portions of this work it is necessary to have the conditions present in these two locations for a basis of comparison, as they are the base locations for most of the materials that have entered into some of the more important experiments in nature and in the laboratory.



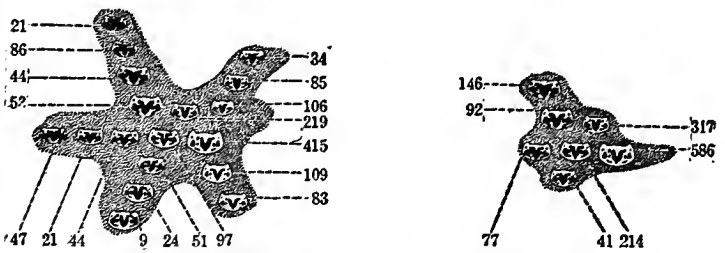
1034 Males. Total 2304. 1270 Females.

FIG. 124.—First census at San Marcos in 1904, showing condition in the pronotal pattern.



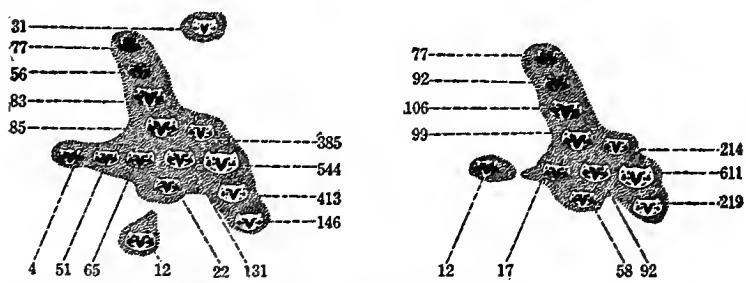
1385 Males. Total 2466. 1081 Females.

FIG. 125.—Second census at San Marcos in 1004.



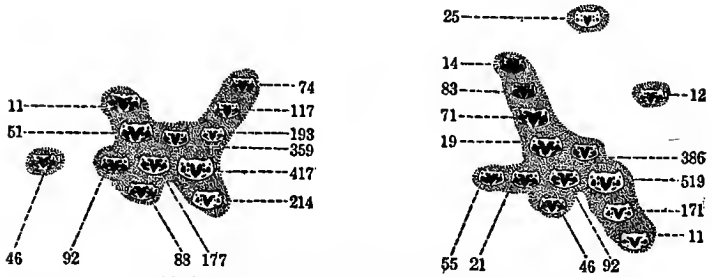
1559 Males. Total 3032. 1473 Females.

FIG. 126.—First census at San Marcos in 1905, showing increased array of pattern conditions and existence of an isolated group in biotype 12.



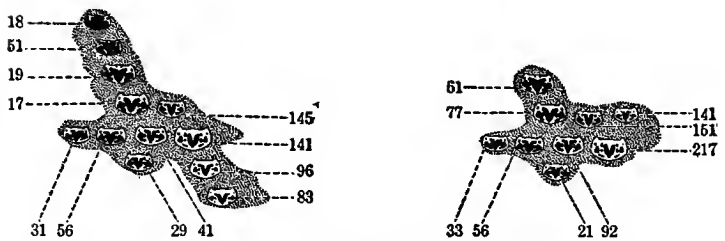
2105 Males. Total 3716. 1611 Females.

FIG. 127.—Second census at San Marcos in 1905, showing condition in the population as a whole and the existence of two isolated groups in both sexes.



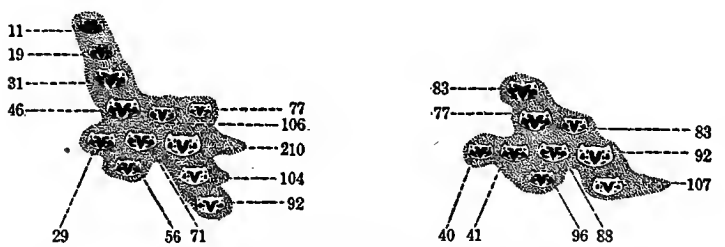
1839 Males. Total 3364. 1525 Females.

FIG. 128.—First census at San Marcos in 1906.



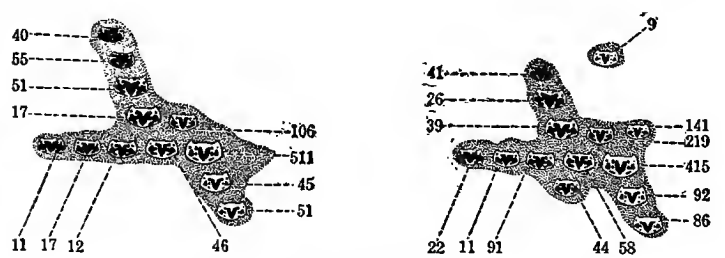
727 Males. Total 1566. 839 Females.

FIG. 129.—Second census at San Marcos in 1906, showing condition of pronotal pattern.



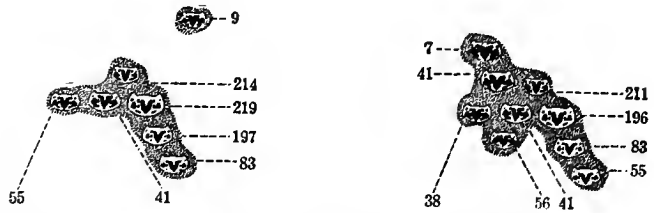
852 Males. Total 1559. 707 Females.

FIG. 130.—First census at San Marcos in 1907, showing conditions in pronotal pattern.



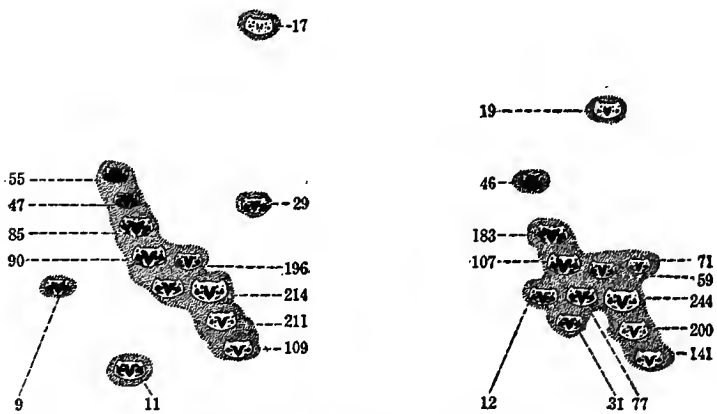
962 Males. Total 2256. 1294 Females.

FIG. 131.—Second census at San Marcos in 1907, showing the pronotal pattern.



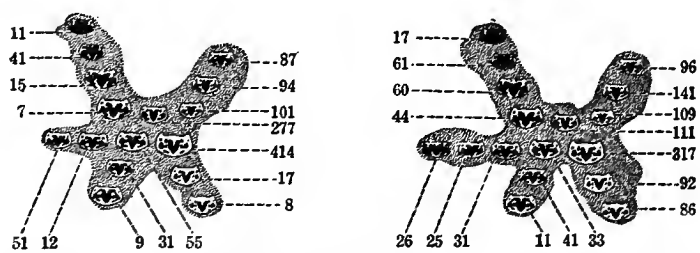
818 Males. Total 1546. 728 Females.

FIG. 132.—First census at San Marcos in 1908, showing the pronotal pattern.



1073 Males. Total 2263. 1190 Females.

FIG. 133.—Second census at San Marcos in 1908, showing sudden change in the population as a whole and sudden appearance of isolated groups in biotypes 6, 8, 12, and 10.



1230 Males. Total 2531. 1301 Females.

FIG. 134.—First census at San Marcos in 1909, showing an increased array in pattern conditions with total elimination of isolated groups present in the population in previous censuses.

Comparison of the two locations is shown in figure 135, in which the outlines of the populations are shown as in figure 111 for *L. multitæniata* for its different locations. *L. undecimlineata,* while conspicuously less widely ranging in its pattern, an "invariable" species, shows the same set of conditions present and acting in the same system, as was seen in the first species, and could the investigation of this species have been followed advantageously for a widely separated set of places most interesting arrays quite different in their composition would have been seen. As it is, in the consideration of the geographic aspects of this complex pattern some indication of the diversity present in many localities is obtained. These, while not presenting the entire series of ever-changing differences that are found in the population, reveal that over the range of the species, as a whole, different locations in space constantly show differences in the array of the pattern presented—instances of determinate variation.

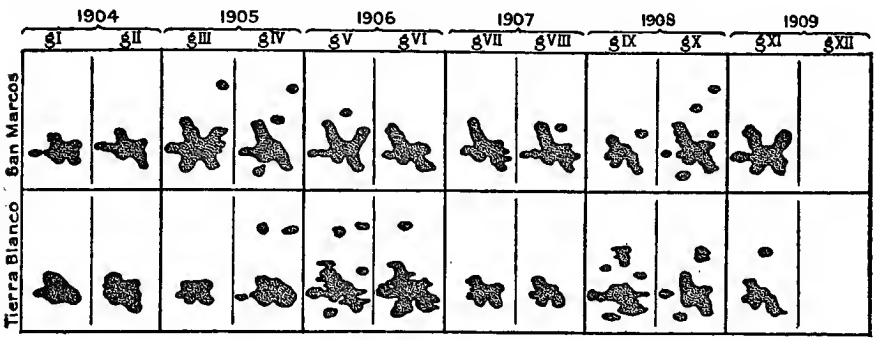


FIG. 135.—Diagram representing the conditions found in the two populations of *L. undecimlineata* in the colonies of San Marcos and Tierra Blanca, during the period of observation. Although the colonies are essentially alike, as far as populations are concerned, there appear to be minor constant differences present in both.

One other instance of the series, namely, *L. decemlineata* at Chicago, for a series of years, will complete the presentation of this portion of the data.

IN LEPTINOTARSA DECEMLINEATA.

In the populations of this species I had examined in Massachusetts, Long Island, and Ohio, I had found that there were present in this character associated conditions that could be grouped into assemblages that were capable of isolation into true-breeding groups, as far as the analysis had then been carried. Further analysis of this condition at Chicago between 1902 and 1907 showed that in the population there were five groups that could be isolated and that would breed true in mass cultures without further selection, once they had been purified. This species is poor in elements of the pattern, none of the extra areas being present, and only the main elements are represented. So, also, the species is poor in possible combinations and is quite distinctive and specific in this respect.

THE CHICAGO COLONY.

There are present at Chicago five biotypes, 7, 4, 8, 9, and 13. In some other locations in the range of this species there are indications of the presence in the

population of other biotype conditions, but these I have not examined or attempted to follow, the localities being too remotely separated from each other and the materials inferior in most respects to those available to me in the tropical areas, where the topography had concentrated the distribution without decreasing the diversity. These conditions, with good transportation in the region, determined the location of most of my work in this direction.

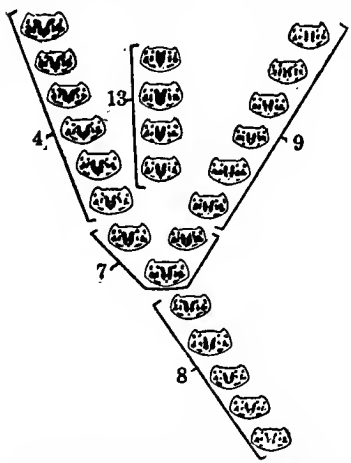
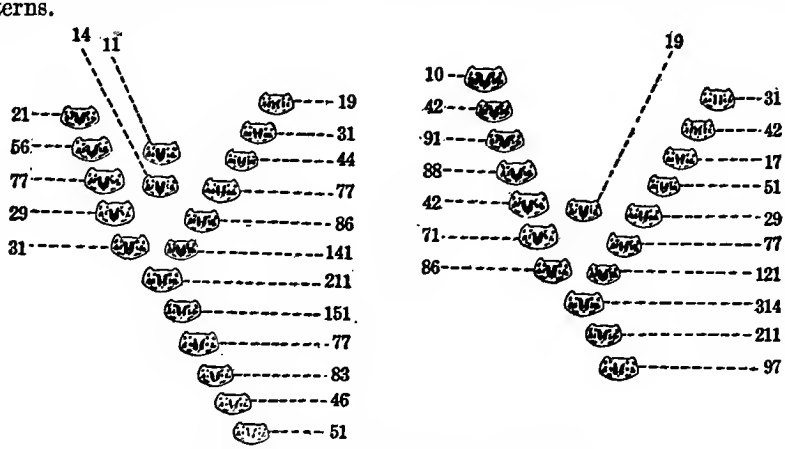


FIG. 136.—Diagram showing biotypes commonly present in *L. decemlineata* as observed at Chicago and the biotypic groups which have been isolated.

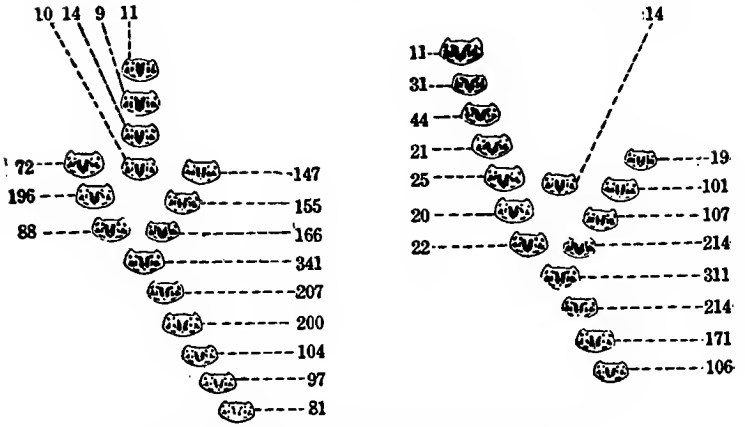
At Chicago, between 1902 and 1907, 10 generations in a restricted location to the south of the city were examined, 2 generations being missed, one in 1905 and one in the early part of 1906. The condition in the population of this species is shown in figure 136, with the arrangement of the different biotype groups in the pattern array. This species is, in part, like the condition in *multitæniata* and in the presence of biotype 13 is like *undecimlineata,* although the presence of the same biotypic conditions in the pattern has no necessary connection phylogenetically, the condition representing solely similar arrangements in the effective forces that produce the conditions observed, and is the sole result of the interaction of the effective pattern-producing agents and the conditions of the medium at the time of the reactions producing the patterns.



1256 Males. Total 2695. 1439 Females.

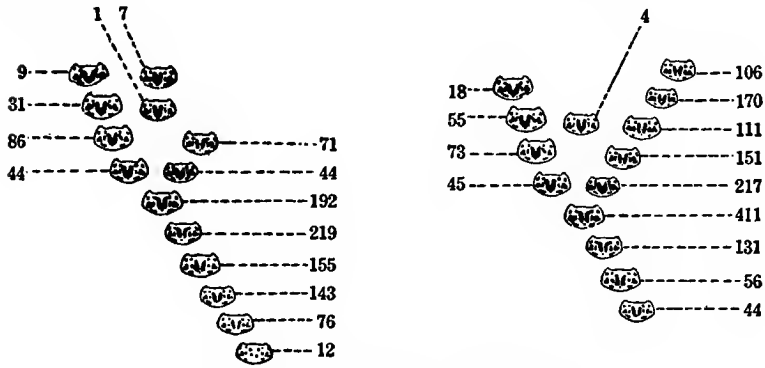
FIG. 137.—First annual generation at Chicago in 1902.

It is not worth while describing at any length the conditions in the different generations examined, the series of determinations showing a restricted range in the array, with not much in the presence of isolated groups in the population, although such occur, as, for example, in the census of 1903 and again in 1905. These are never far separated from the general mass of the population, but are the extreme members of some of the biotypes, between which and the general population there are no intermediate conditions presented. The array shown in the censuses that are given in figures 137 to 146 show in principle the same conditions that were found in the former species.



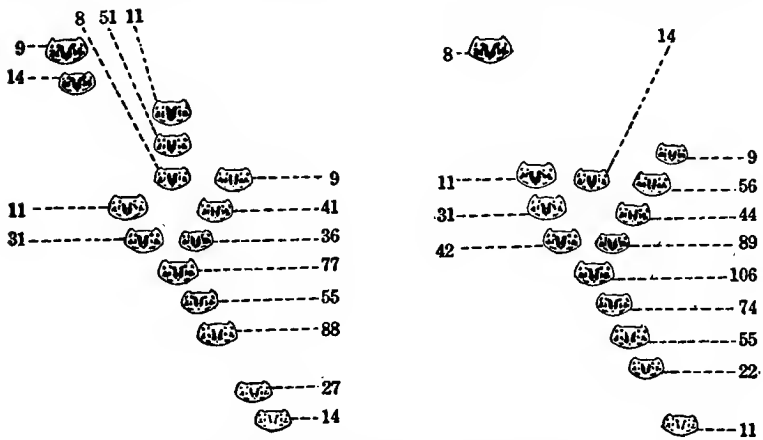
1898 Males. Total 3329. 1431 Females.

FIG. 138.—Second annual generation at Chicago in 1902, showing the pronotal pattern.



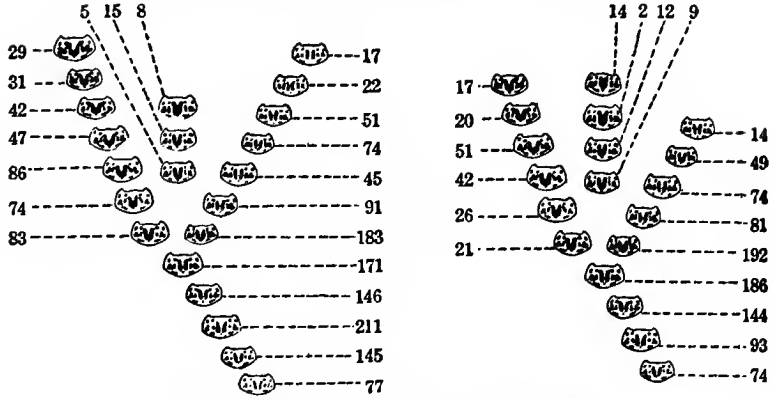
1090 Males. Total 2682. 1592 Females.

FIG. 139.—First annual generation at Chicago in 1903, showing the pronotal pattern.



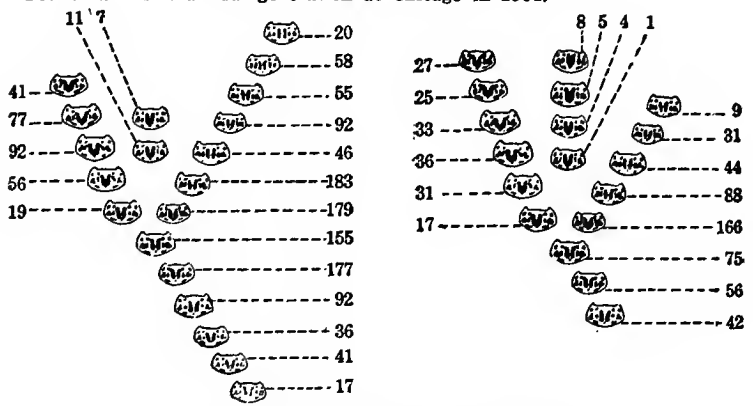
482 Males. Total 1054. 572 Females.

FIG. 140.—Second annual generation at Chicago in 1903, showing condition in population and existence of isolated groups in biotypes 3 and 4.



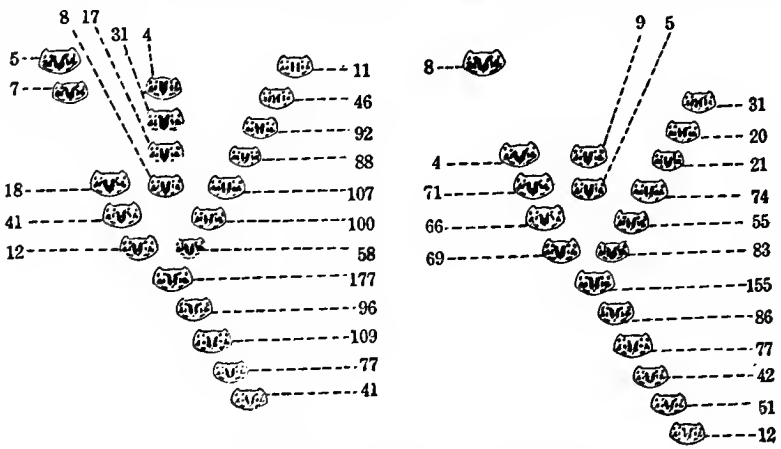
1653 Males. Total 2774. 1121 Females.

FIG. 141.—First annual generation at Chicago in 1904.



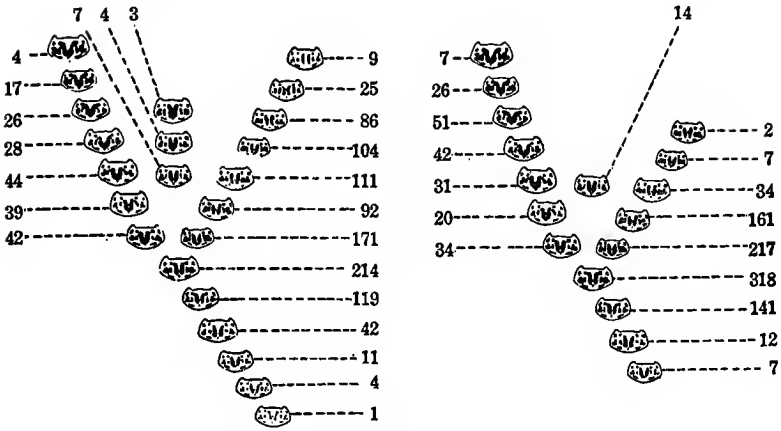
1454 Males. Total 2152. 698 Females.

FIG. 142.—Second annual generation at Chicago in 1904, showing the pronotal pattern.



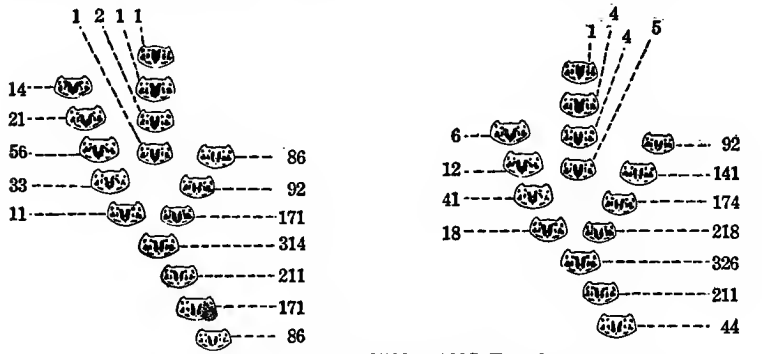
1145 Males. Total 2084. 939 Females.

FIG. 143.—First annual generation at Chicago in 1905, showing conditions in population and existence of biotype 4 as an isolated group in both sexes.



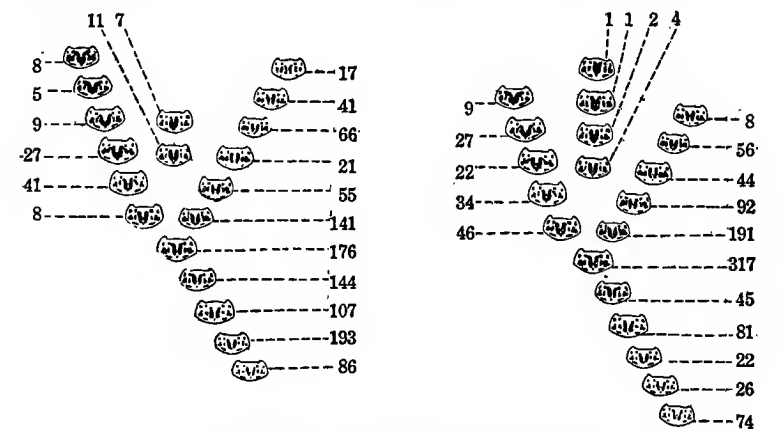
1203 Males. Total 2327. 1124 Females.

FIG. 144.—Second annual generation at Chicago in 1906, showing the pronotal pattern as far as observed.



1271 Males. Total 2568. 1297 Females.

FIG. 145.—First annual generation at Chicago in 1907, showing the pronotal pattern.



1163 Males. Total 2265. 1102 Females.

FIG. 146.—Second annual generation at Chicago in 1907, showing the pronotal pattern.

SOME RESULTS OF THESE ANALYSES.

The analysis of the condition in the population covering a series of generations in the same location has not often been attempted, and when it has, not infrequently the outcome was to arrive at incorrect results, owing to the short time that the observations were continued, or to the wide gaps that intervened between different determinations. One of the best examples of this is the experience of Kellogg with *Diabrotica soror* in California, who examined the population of this beetle, finding certain conditions in the population as expressed in the variations of the elytral pattern, and later, with an interval of several generations, a second set of determinations gave results that looked like movement of the population, which was strengthened by the determination in one or two succeeding generations without change; and so the conclusion was reached that the population had changed in one direction with respect to this character, between the first and the second censuses, and the results were described as determinate evolution. Later determinations showed that the population had moved back to the first position with respect to the same wing characters. What happened in this instance is that the lack of continuity in the observations gave false appearance in the determinations taken at random times. Out of the data that I have had at my disposal, any number of instances of this sort could be realized by precisely these methods.

The data that I have presented in the preceding pages would, by the statistician and his measuring-stick, be massed by force into curves of error, interpreted in terms of some hypothesis, and then put forth as "proof" of the same hypothesis. So, also, the faunistic and ecological worker and the geographic variation worker could and would utilize the arrays presented as examples of the direct action of environment upon an extremely labile species, and derive various agents that may or may not have been operative in the actual conditions in nature. These plausible relations, easy to create, soon become fixed in the minds of the worker and are soon "established facts" in support of the especial proposition with regard to the action of geographic influences.

Some, no doubt, conceive of the population in terms of minor groups which the census does not reveal, and can therefore be interpreted upon the basis of the presence in the population of numerous elemental conditions "without intergrades," but whose "fluctuations" overlap and thereby produce the appearance of continuity, wherein there is no real "continuity" excepting in the "fluctuations of the minor groups." No census can show anything with regard to the truth of this proposition.

With these materials I have gone carefully over the series and have applied the census methods, but nowhere have I been able to discover that the real nature of the materials and of the processes at work was even indicated. These are problems for the laboratory and the experimentalist and not for the statistician.

I, for one, do not care to discard either the method or the point of view of the statistician, as some would do, because the method does give information concerning the mass of the population that is of real value in the attempt to experimentally analyze its constitution and the processes at work; but more, it gives accurate information that could never be provided by the laboratory and the experimental garden with respect to the sum total result produced in and the aspect of the population after it has been subjected to the operations of nature.

The methods here employed, with the information of the constitution of the character and the experimental analysis of it have given the opportunity to understand rather well the meaning of the conditions in the natural populations that the censuses have revealed, and this is of value in the analysis of some of the problems of the production and origin of species and minor groups in nature and in experiment.

I have been especially interested in the constant presence in the population of "definite variation," which is true not only in the single population, but in each location there is an unmistakable place response in which the population "varied" in the same direction as the result of the conditions generally present in the population and in the medium. I do not recall at present anyone who has attempted continuous analyses of these responses in any population in nature; rather most of the instances that are in the literature are the plausibilities of records from few specimens or from determinations made at one time. Nor do I recall that the use and recognition of the principle that Darwin so clearly saw and believed might be of so much importance in the evolution of organisms has been often mentioned or considered. There is an abundance of argument concerning the production of the "directed" conditions which are supposed to exist by "selection," but almost no attempts at the analysis of the situation. Some few, as in the crab *Carcinus,* investigated by Weldon, gave information that is at once suggestive and at the same time too incomplete to be of much service in the analysis of the problem, and the results can be interpreted on any basis one wishes. The incomplete facts as determined show only that changes of some sort were in progress, either permanent in character or temporary, ontogenetic or germinal, due to selection, environment, or any other cause one wishes to introduce. The unquestioned fact of change existed, but was not analyzed.

There is no question but that the conditions as presented in the populations represented here show this definite place "variation" in the population as a whole in this complex character, and the census methods employed leave no doubt of the reality of the conditions revealed. If these responses in the population of a place as a whole to the conditions of the habitat have any meaning with respect to the production of heterogeneity in nature, this set of materials provides at least one opportunity to test it out. The populations as found in the determinations and the general place tendencies in the population may be produced by one of two general conditions; either the place response may be due to a real germinal difference in the race, or it may be due to the ever-present action of the conditions, influencing the soma in a definite direction in its "fluctuating variations," or it may be due to the presence in the location of influences that eliminate a proportion of the population possessing certain characters, so that the maturing population presents only the aspects of the portion thereof that was able to escape the eliminating agents. A further possibility is that the conditions seen in the population are the product of the combined interaction of the character of the combining materials and the conditions of the medium in the habitat.

If the condition in the population at any location is a permanent one, then the differences that are presented may well be the basis of further differentiation and in the end result in the production of a distinct localized race and

add another element to existing heterogeneity of race and form in nature. These questions I have put to an experimental test as an aid in the further analysis of the problems of heterogeneity in complex characters.

PATTERN IN DIFFERENT HABITATS.

In my 1906[1] paper, under the terms "place variation" and "geographical variation," I have discussed the differences found in the population in several species of beetles belonging to this genus, using the current biometric methods of expression and interpretation. Also, the experimental data then presented were purely of the sort indicated by the quantitative, qualitative aspects of the problems, although I then knew well enough that the whole aspect of the problem would soon change its setting and method of analysis. From the point of view of the presentation in the 1906 paper nothing more is to be added to the data and the conclusions there presented, and nothing further is to be expected by continuation of the method of examination. Analyses had shown that all the materials examined presented the phenomena of place variation in different degrees, and the same species in the same place showed different variations, depending upon the season. These biometric results and the legitimate deductions to be derived from them, with the experimental attempts to fix mere quantity as measured statistically, while showing the presence of the condition with regard to the phenomena and their difference in the several species examined, lead to some points of view and conclusions that are essential to tie back to as a part of the general problem that receives further analysis in this report.

It was there recognized that on the basis of the results presented place variation was a generally present phenomenon in the organism examined, and also in general that it affects every portion of the animal (p. 103), and that not all animals are alike in their variability. The methods employed led to the conclusion that the "variations" were not germinal, but somatic fluctuations, the product of the varying conditions of the environment (p. 102), and that some of these conditions of the amount of pigment or other characters were in favorable instances to be rather accurately tied in interpretation to conditions in the environment. The fact that in experiment the increase in the amount of decrease was easy to produce, and had been produced in many experiments, led to the conclusion with respect to the permanence of place variation that it was not, as far as the evidence then went, permanent and a probable factor in the production of evolution changes (p. 102). A possible correlation between the production of extreme variations, simultaneous with the occurrence in the environment of divergent conditions, was noted, its possible action indicated from actual examples, and its bearing upon the relation of these productions to varying conditions, suggested lines of experiment (p. 104).

With respect to the condition as there measured by statistics, the conclusion is valid as expressed, that the conditions thus determined were not permanent, capable of fixation in experiment, and it is further true that many of the differences in the array of the measurements are mere ontogenetic exaggerations and nothing more, due to the action of incident conditions at appropriate periods in the life of the individuals. At present I fully concur in the result expressed in

[1] An Investigation of Evolution in Chrysomelid Beetles of the Genus Leptinotarsa. Carnegie Inst. Wash. Pub. 48.

1906 as to the permanence of this sort of "variation" when determined in this manner. Attempts since 1906 have been as positive failures in this respect as were the earlier ones.

Biometrically the assumption is made that the material is homogeneous; in reality it is not so, and eliminating by proper methods the larger portion of the somatic variations, the proposition is still true that any instance of statistically determined amount is not permanent and does not become fixed by continued selection, due to the fact that the statistical determination does not choose identical conditions in the character. No better demonstration of this could be asked for than that presented in the history and improvement of the sugar beet. The area of the pigment exposed on the pronota of my material may well be equal in area or proportion of surface exposed, but the pattern conditions presented, the array of active agents, factors, and determiners present and that are brought into this array gives so extensive opportunity for heterogeneous conditions that the real nature of the series is constantly shifting and is hopelessly confused, so that the result statistically is to end with the "conclusion" that the "variations" are somatic and so not inheritable. They may be so or not; the method has not been able to test the point. I have seen series in which the conditions present were known to be accurately inherited and recognizable by the arrangements in the pattern, but the biometric method resulted in a hopeless array of ranging areas of pigment, which could not be fixed. It is this recognition of the results of the confusion of real conditions under the blanket of biometrics that formed the transition from the former situation to the present one. The results attained in this first set are correct as far as they can be with the method employed, and were carried as far as it was possible to go in nature and in the experimental analysis of the problem of place variation.

As has been shown in the preceding pages, this pattern is not a haphazard array of colored areas, but an accurately constructed system, in which the color is the end-result of many agents, and from this point of view quite different conditions are conceived of in the population as the cause of the heterogeneity found, and so the phenomena of place and of geographic variation take on a new aspect, becoming capable of analysis along new and profitable lines. This is not the place to deal with the gametic constitution of the materials used or of the factors that have been recognized as present and acting in the population in this complex system.

It is from this point of view that the phenomena of place and geographical "variation" deserve analysis, so that the result will be a determination in terms of effective agents, internal and external, in the production of the results observed. Further, the examination will answer accurately questions as to the actual, permanent, germinal differences that may or may not exist in the separate portions of a widely distributed race, and in experiment these differences can be tied to combinations of environmental conditions that exist in nature, so that in the aggregate a more complete understanding and picture of the conditions in nature will be produced. Out of investigations of this sort must come the rational explanation of the origin and meaning of the facts presented by closely allied, locally placed isolated races, as for example the Achatinellidæ in the Hawaiian Islands, as described by Gulick, and many other similar instances in nature. The explanation of the origin and the experimental duplication of

them will rest upon the interaction of the conditions of the medium and the character of the factors or determiners, and their location, association in the system, and capacity to react uniformly under a given set of surroundings and associations. A most desirable result of the method of analysis and the determination of the interacting agents will be the passing of choice, selection, purpose, utility, isolation, segregation, and the rest of the array of "biological explanations" current in the literature as an "explanation" of the position in closely placed habitats of forms, much alike but sufficiently different in minor aspects to give true taxonomic value to them.

Two main questions arise in this connection and can be successfully attacked through experimental analysis: (1) In a given "species" or "race," in any or all its habitats, does the actual germinal condition of the race differ in successive generations, or is it constant throughout for the location and form? (2) In different habitats does the constitution of the race change in constant, recognizable gametic differences? These are tests that should be made upon species or races that are considered by the best of taxonomists as uniformly good species, which are real things in nature. Recognized geographical races are to be excluded from this analysis, which is planned to reveal, if possible, whether in nature, separation into habitudinal groups of naturally uniform race may not begin in local areas and be the result of processes that can be determined and utilized in experiment.

In the investigation of these problems I have used three methods of attack. (1) The introduction of stocks from one colony into other isolated locations and the watching of the following generations and the recording of them, to determine what they did in terms of the census-taker in the new locations. This will give rather useful data with regard to the readiness of the stocks to respond to different conditions, and also the direction and extent of the response. (2) The transportation of samples of the materials taken at random in the population from the locations under observation to some central location, where all are reared in isolation under one set of conditions, and in this manner can be tested the reality and permanence of the differences in the populations from different locations. If the samples are random, and if the breeding is in group culture, with no selection in the matings, then the result will be an accurate demonstration of whether the differences present in the original location are able to persist alongside of the differences found in other locations, when both are living under the same conditions. This I have tested by taking the materials to Chicago and growing them under conditions that were uniform and neutral to all of the complexes that were under examination. Under experimental conditions of this sort the result will be an accurate measure of the permanency of the character of the material in both its germinal and somatic capacities to be reproduced in like values in successive generations. (3) The analysis of the conditions of the population with pedigree analysis, especially of the extreme conditions and of the most common condition. Collectively the three will give answers to the problems investigated.

EFFECTS OF TRANSPORTATION INTO A NEW HABITAT.

In this method of testing the permanency of the array shown in a local population, the results that have been obtained in transplanting materials from the colonies at Chapultepec, Tlalnepantla, and Chalcicomula will serve to show the

general type of results arrived at in *L. multitæniata,* and in the same way with materials from Tierra Blanca and Campeche the conditions in *L. undecimlineata,* which will be fairly illustrative of the method and principles. In this work it would have been fortunate if the opportunity had been available to have used cages in some of the habitats, to contain materials taken to that point from some other, so that the tests would be made more accurate and much more critical. One method employed was to find isolated locations in which the species was not living, stock them with food, and then introduce the materials to be tested. This was not so difficult in southern Mexico, where the topography lends itself admirably to the needs of problems of this sort. Most fortunate conditions of isolation were found ready or easily created in the southwest portion of the valley of Mexico. At this point in the valley a huge surface lava-flow has broken out of the sides of the volcano of Ajusco and flowed down over the sides of the valley, covering the whole mountainside from Contreras and San Angel on the northwest to the Rio San Buenaventura on the south and to Tlalpam on the northeast and east. Although recent in geologic time, there had been sufficient erosion and denudation to provide the region with a rather well-developed but peculiar flora, and, most important, the forming of pockets of soil in the much-broken surface of the flow that could be utilized as locations for growing the food for these animals for the introduced colonies. In the area as I found it there was no food for these animals and none of them was found living in the area, due to the fact that the agencies of dissemination in the valley were entirely against their introduction. The pedregal had become inhabited by plants and animals from the higher slopes of the mountainside that had been carried down by the wash of the storms. No roads traversed the area, and only a few footpaths crossed the edges thereof, so that introductions by transportation were trivial factors. The added character of the area that it was of no use as a grazing-ground made it immune to the introduction of *S. rostratum,* the food of these animals, by grazing cattle, one of the most common and efficient means of disseminating the plant. By going into the area from 1 to 2 miles, conditions of complete isolation were obtained, and with considerable labor and trouble the proper locations were obtained. These had, within certain limits, the same general set of conditions, which, located as they were, were not greatly different from the location at Chapultepec. It was found in practice that the dissemination from the colonies was slight, owing to the character of the bare, dry lava-flows about each of the locations chosen, so that if any of the beetles did wander from the colony the chances were immensely against their ever gaining the next one in the maze of pits, cracks, caverns, bare, hot rock surfaces, and so on, that completely filled the area, so that the random wandering of one of these animals would with large probability end in elimination and not in reaching the next location, even when only a few hundred yards away. So broken and bad was the surface, so cut by pits, huge, deep cracks in the lava, that passage of the portion used, on either horse, mule, or burro, was impossible, while foot travel was slow and tiresome.

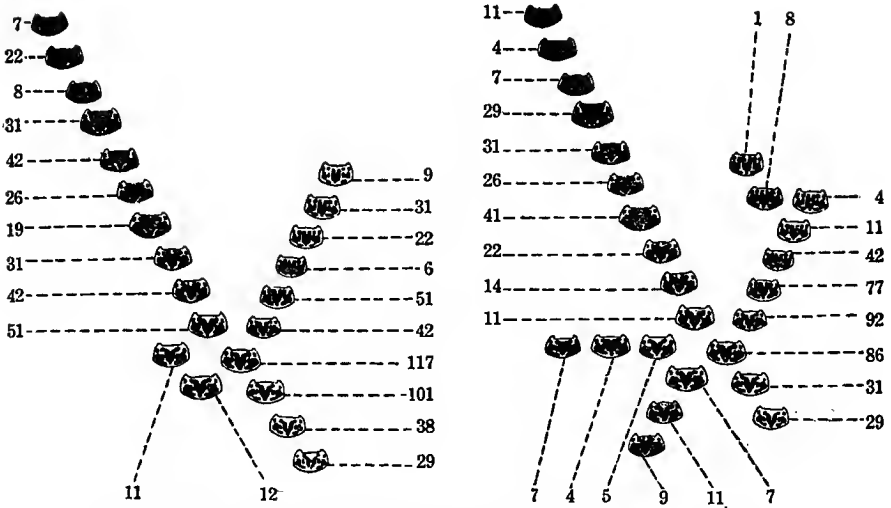
The first tests were begun in 1905, when 10 good locations were found, located in the midst of the pedregal and isolated by distances varying from 300 yards to a mile apart. The majority of these locations were depressions in the face of the flow into which denudation from above had washed accumulations of soil, in some instances to a depth of several feet. The areas were mostly

oval or roughly so in outline and on the average were about 60 feet in diameter. They were denuded of the original vegetation, sown with the seeds of *S. rostratum* from the valley, and in a few days the location was ready for the test.

Early in June 1905, I introduced into the locations prepared random samples of *L. multitæniata* from the colonies at Chapultepec, Texcoco, Tlalnepantla, and Chalcicomula, and all thrived and became self-sustaining with the exception of the introduction from Texcoco, which died off, owing to failure of the food-supply. This was the result of too shallow soil in the spot chosen. In each test the same method was followed as was employed in the natural colonies, that of making a census of the population twice each year. At the time I did not know what the condition in any of the colonies would be shown to be, but I was certain from previous experiences with the biometric methods of study that the problems had to be attacked, and that this was the best method in this one direction, so that in the series the test and the study of the conditions in the colony went on simultaneously, a combination that has obvious advantages. The other locations were utilized for other tests of the same sort that were made on other species. Observations were continued at the three locations, two determinations in each season from 1905 to the end of 1908, eight generations in all. One exception in the census determinations was made, namely, the dropping of the rating of the amount of the pigment present in each of the pattern types shown, only the types being recorded in the test locations, because it was thought that the persistence of the types was the point to be determined and not the amount of "fluctuation." In presenting the records of the tests in this paper the sexes are not separated, although they were in the original determinations. There are no sex limitations or dimorphisms with regard to this pattern, and the mixing of the two into one population statement does not, as far as is known, hide or alter any of the conditions of the test.

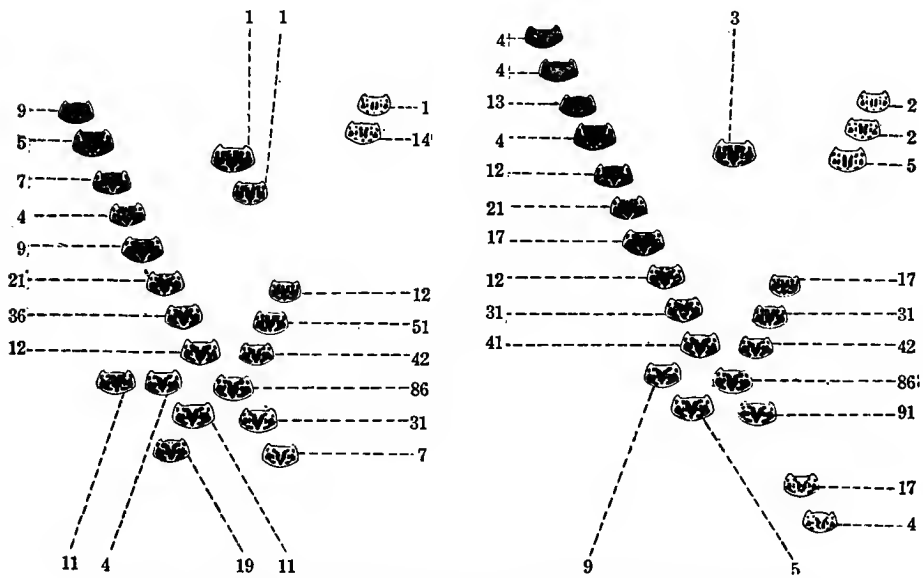
THE CHAPULTEPEC COLONY TEST.

The character of the introduction of the stock from the Chapultepec colony is exhibited in figure 147 in the array of the population for the first generation of the year 1905, and were the overwintering members of the previous year's last brood. The chance distribution of the parent test group is shown in the figure in comparison with the progeny of the group at the normal location. At the test location the whole population was allowed to take part in the operations of reproduction throughout the series, so that the test series was treated, in all respects, like an independent isolated colony. The results of the test are shown in figures 147 and 148, and do not reveal change in any essential in the test location. The two locations were not entirely identical, and it was thought that there might be some difference in the new location, due to the change, and some little was found, but it is not at all certain that it was the product of the unlikeness in the conditions, and might as well have been due to altered relations of agents in the gametic complex as to the environment. There was certainly nothing at any point to indicate the environment as the sole active agent in producing the observed departures (figs. 147-151).



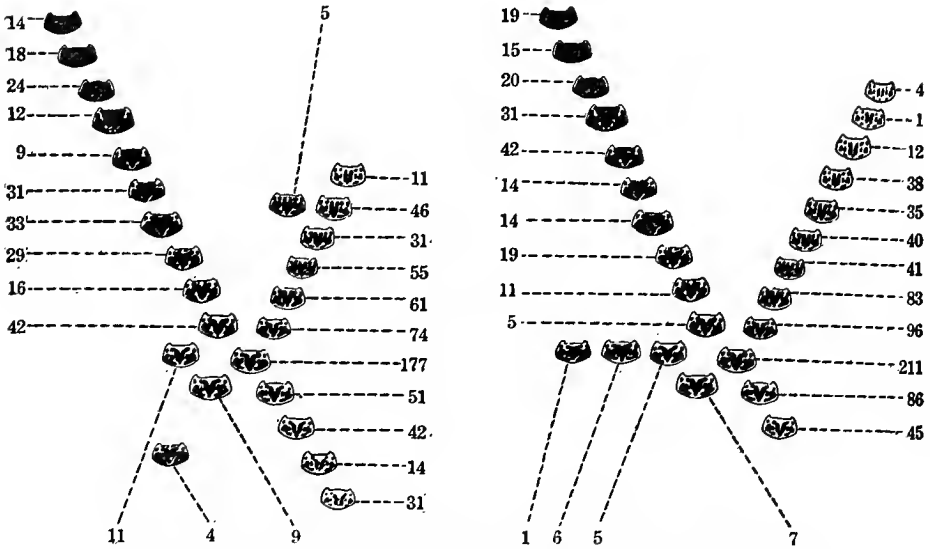
Population 748, First Generation. Population 620, Second Generation.

FIG. 147.—Census showing condition in the test colony of Chapultepec in 1905.



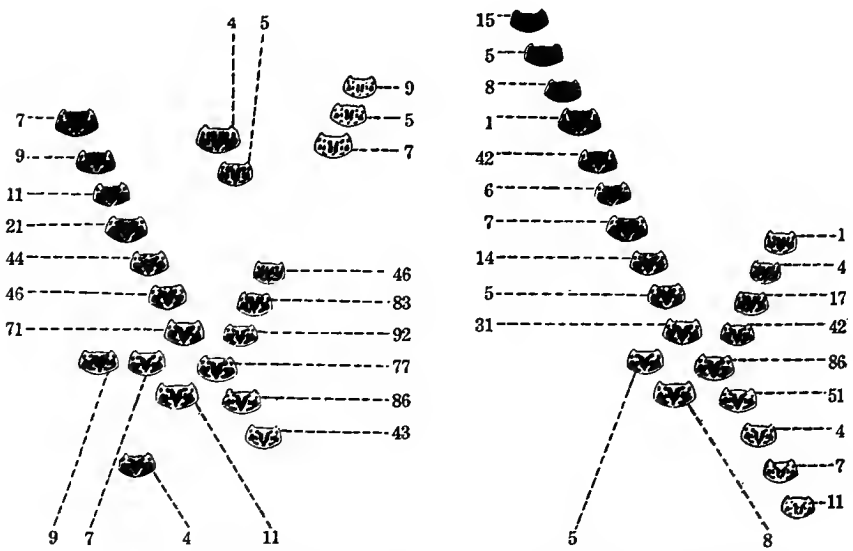
Population 394, First Generation. Population 473, Second Generation.

FIG. 148.—Census showing conditions in the first and second generations in 1906 at Chapultepec test colony.



Population 850, First Generation. Population 901, Second Generation.

FIG. 149.—Census showing conditions in the first and second generations in 1907 at Chapultepec test colony.



Population 697, First Generation. Population 370, Second Generation.

FIG. 150.—Census showing conditions in first and second generations in 1908 at Chapultepec test colony.

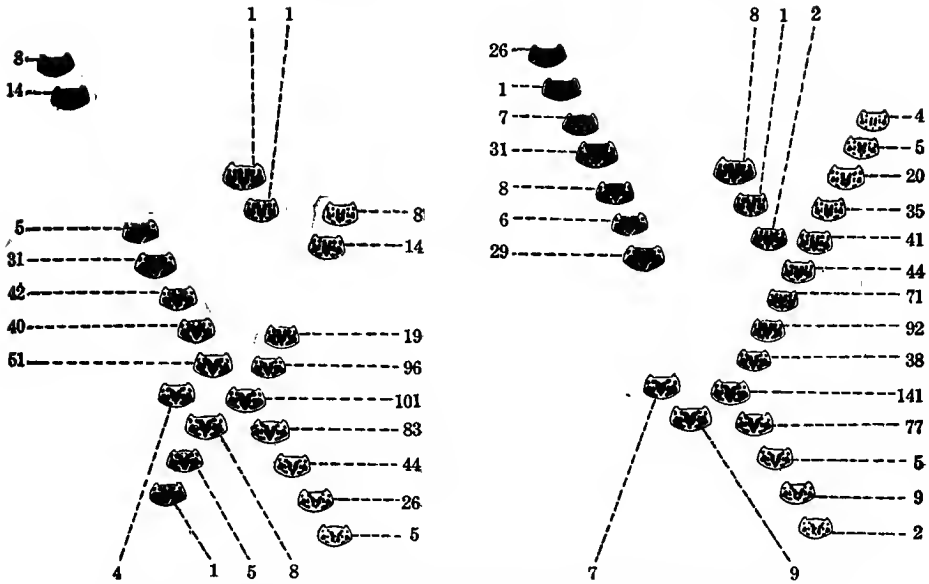


FIG. 151.—Census showing conditions in first and second generations in 1908 at Chapultepec test colony.

THE CHALCICOMULA COLONY TEST.

About the middle of June of 1905 a representative culture from this location was introduced into a test location about a mile from the Chapultepec test, and there thrived well and gave in the following generations a well-defined group that was constant in appearance. In figures 152 to 156 are shown the results

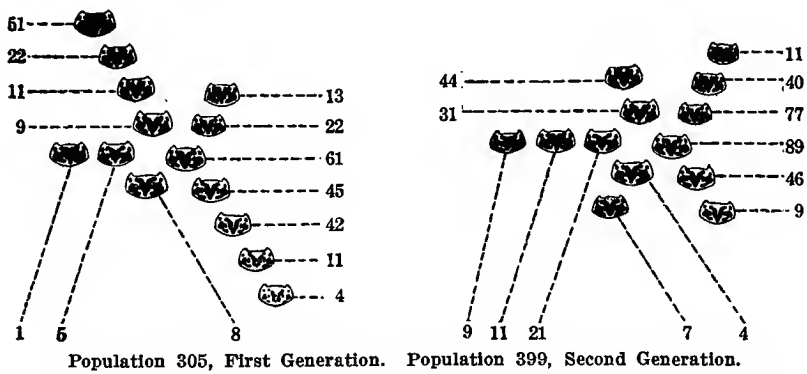
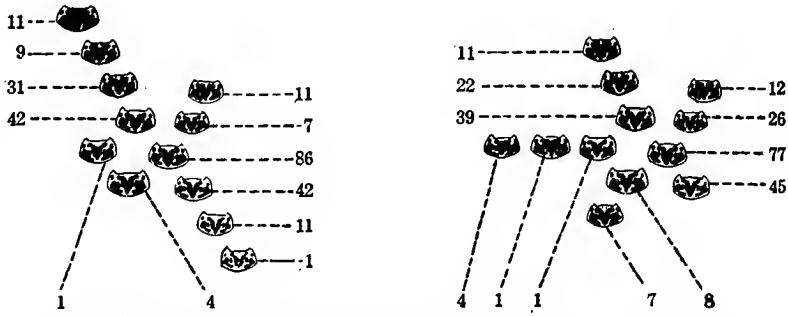


FIG. 152.—Census showing conditions in first and second generations in Chalcicomula test colony in 1905. In the first generation the introduced generation are indicated along side of their progeny.

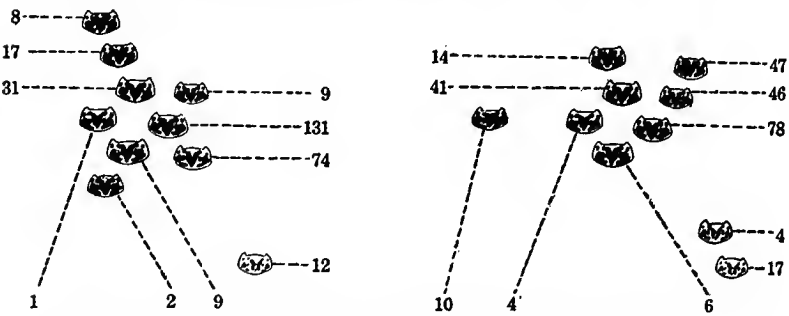
of the tests in these locations, with the summated generations in the original colony for the corresponding generation and time, with the result that in the new location the test colony remained well within the range of the population in the parent location during the same period. In the original location the population

was characterized by the absence of the factors in the make-up of the population that produced biotypes 1, 2, 3, 10, 11, and 12, and these were not present in the introduced group and did not appear during the test, whereas in the test of the



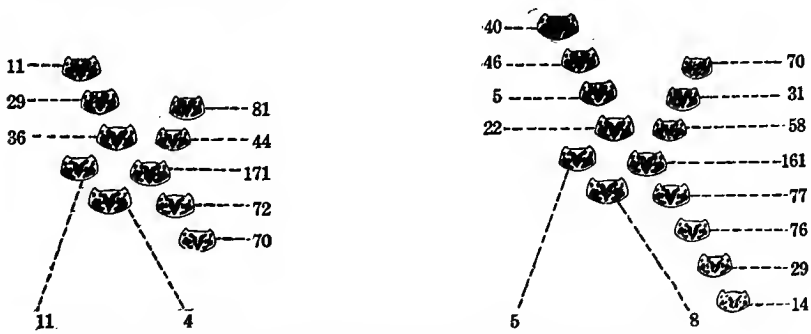
Population 256, First Generation. Population 253, Second Generation.

FIG. 153.—Census showing conditions in the first and second generations in 1906 at Chalcicomula test colony.



Population 294, First Generation. Population 267, Second Generation.

FIG. 154.—Census showing conditions in first and second generations in 1907 at Chalcicomula test colony.



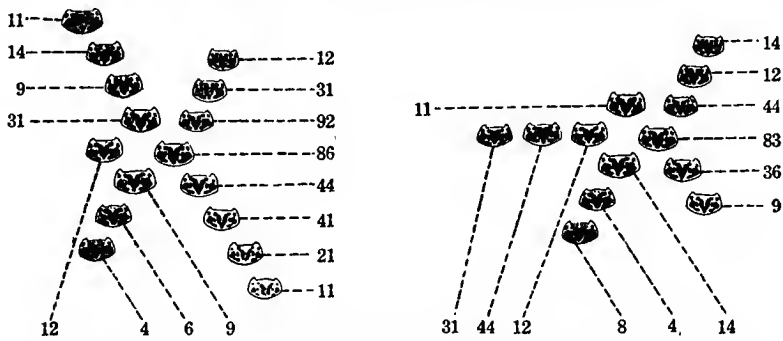
Population 529, First Generation. Population 642, Second Generation.

FIG. 155.—Census showing conditions in first and second generations in 1908 at Chalcicomula test colony.

Chapultepec location under the same conditions all of the biotypes were present and none were added or lost. The same is true of the test of the Chalcicomula colony; none was added or lost in the test location. The test in this instance

represents considerable change in the environment to more optimum conditions, nevertheless the change did not put into the test series the agents to produce the conditions in the pattern that are not present in the parent location to bring it up to the same range that is found in the species native in adjacent portions of the valley of Mexico.

These two tests show that the condition in the population in at least two locations is not temporary, to be shifted about at the will of the oscillating environment, but is relatively stable and the product of the present and workable factorial composition of the population, and of no other source. The unchanged condition shown by the tests of the Chalcicomula colony is in all respects the result of this, and if by any means there had been introduced into the test series the *multilineata* form-factor, the *melanothorax* pronotal pattern-factor, an extension factor, and the factor and determiner for the anterior



Population 434, First Generation. Population 322, Second Generation.

FIG. 156.—Census showing conditions in first and second generations in 1909 at Chalcicomula test colony.

marginal spots, there would have been produced in two or three generations all of the missing biotypical conditions in the pattern.

OTHER TESTS.

As stated in an earlier place, a test was made with the colony at Tlalnepantla, at a location in the pedregal about 0.75 mile from the Chapultepec test location, but with identical results, which I have not thought it worth while to burden this paper with. The population in the Tlalnepantla location showed some interesting differences from the other locations in the valley at that time, especially in the absence of biotype 3, in which the operation of an extension factor plays a considerable rôle, and this seemed to be entirely wanting in that location, and the test series showed the essential conditions that were present in the parent colony. At diverse times tests have been made for two or more generations with the stocks from other locations at points in the pedregal in the manner indicated. Thus the location at Puebla was tested at Esperanza in 1906 and 1907, Ometusco in 1907 and 1908, Tlascala in 1906, and in none have I thus far obtained any but the same kind of results as here shown.

In this portion of the study I have been careful to use only the materials of this species that are well within its central range, and at no point in the range employed is there anything like geographical races, so that the tests have been

made upon the portions of a natural uniform species, variable to be true, and complex in its make-up, and for that very reason probably a good one upon which to test out some of these points, showing as do the results that in a uniform but variable species (in the taxonomic and faunistic sense) the materials taken at different points over its natural range are not of necessity identical in their germinal constitution, but that the conditions differ in neighboring placed habitats, and that these gametic habitudinal differences are permanent.

Similar tests were made with less satisfaction, owing to practical difficulties in the years 1905 to 1907 in some of the steep-sided and well-isolated ravines that are found in the valley-head above Maltrata. These ravines are the location of small streams falling precipitously over the edge of the Mexican Plateau to the level of the Maltrata Valley. The location was much more difficult to operate in, and the results were not so interesting, owing to the all too frequent damage to some of the locations by heavy torrential rains that come in the rainy season, often washing everything before them in some of these ravines, and one can not predict when one of these will strike the chosen spot.

The outcome of the tests that were made was in all respects identical with those on the pedregal in the valley of Mexico. Of most interest were the results of the series from Chapultepec, Chalcicomula, and Tlalnepantla, none of which changed in any respect during the time that they were in the test locations, giving uniformity of action in all.

One point is of practical importance in this connection, namely, in placing these colonies for testing the condition in the population, it must be as certain as possible that the location chosen for the test shall not be such in its conditions that they will serve as a modifying agent and so give confusion in the determinations. I used all of the precautions that were possible to avoid this, and seem to have been most fortunate in my selections, not one of the test-series locations showing anything in the way of changes in the population that could in any way be regarded as a direct modifying action of the environment.

TESTS WITH L. UNDECIMLINEATA.

The populations of this species at Tierra Blanca and San Marcos are apparently not sufficiently different to provide much in the way of contrasting conditions for testing, so that the permanency of the condition in the pattern was tested in this species by taking some of the population from the Tierra Blanca to the region of Campeche, and from a point near Campeche to the Tierra Blanca in 1908. The population in the two locations apparently differs permanently in several respects. The chief difference between the two is the absence in the Campeche location, near Seibacabecera of biotypes 4, 5, and 9, with the preponderance of the groups 6*a*, 7, 13, 14, and 15, making a population in which there was little breaking in the continuity of the array. The Tierra Blanca colony, on the other hand, shows the 6*a*, 13, 14, and 15 series weak, and in any given generation separated by large gaps. The stocks were interchanged in May 1908, and each put in a location not in contact with the original colony, but isolated from it as well as the conditions in each location would permit. The samples introduced into each location were fairly random. I did not see the colony again until the winter of 1909-10, when a portion of the colony at Tierra Blanca was probably in the ground in æstivation, but enough

remained upon the plants to make a fairly satisfactory analysis of the population, while the conditions at Seibacabecera were more satisfactory in this respect. In each some three or more generations had passed since the introduction, and at each location there was the probability that some of the native population entered the introduced colony and bred therewith. In spite of this, in the changed locations involving considerable climatic differences and the most probable interbreeding with native stock, the census made in the locations showed that in the time elapsed and with the combined action of the slightly changed conditions and the mixing with local conditions, the two introductions had changed but little if any in the period of the test. I did not see the Tierra Blanca location again until the winter of 1911-12, when the adverse conditions had driven two-thirds of the population into the ground for the dry season; but of these that remained, or that were recovered from beneath the débris on the surface, it was evident that a considerable mixing or environmental effect had been produced. This was indicated by the abundant presence in the population of biotypes 4, 5, and 9, that were only represented by traces in the previous census and that were wanting in the original location. The companion test location has not been visited since 1909, owing to the disturbed political condition of the country.

The conditions observed in the Tierra Blanca test location are certainly not the product of the external conditions, because conditions in the pattern-system, represented by biotypes 4, 5, 6*a*, and 9 are the known product of the presence or absence in the gametic system of exact agents, and at no point in all of my experiments or observations is there the least evidence that conditions in the environment, especially so little different in the two locations, could or had ever produced these changes in the pattern of any group. It is certain, therefore, that the change in the condition of the test colony, which was not and could not be perfectly isolated, had resulted from the incoming of native materials and the incorporation of the missing agents into the introduced group to produce the absent biotypes. So likewise other near locations in the region were showing the effects of the introduction by the increased numbers of the biotypes most characteristic of the Campeche stocks. These crude operations in nature show the permanence of the condition in the pattern in the different locations, regardless of the conditions of the new location, provided that the location does not have conditions that are intense enough to act as agents in directly producing germinal changes. In instances of this sort the action of the introduced colony is always different from that seen in this series of tests.

At one time or another, to different degrees, all of the stocks that I have used in these investigations have been tested in this manner, and in no instance have I found that when the test was made from the point of view of the constitution of the character did the condition change when the habitat was changed within reasonable limits, which is a very different ending from the condition found when the permanency was tested in terms of the statistical value of pigmented area present, when the conditions were not permanent in any instance tested. This is due, of course, to the fact that the area, or the measured biometrical character in general, is not the character, or a character, in the individual or the race.

In the materials that I have used it was necessary to establish the permanent nature of the differences that are shown in restricted locations in nature for two

reasons: (1) It is all too commonly assumed that natural species and materials in nature are "pure," by purity being meant uniformity of action and homogeneity of constitution, which in my experience in using materials that are wild in nature is not true in any instance thus far found. There is taxonomic purity, or limitation to the range of the characters present, and this is a real, permanent condition in these natural species, but within these limits the population shows all degrees of diversity in its composition and in the factors and agents present, so that for accurate investigation it is necessary that all materials from nature be reduced to homogeneous conditions before they are used in the conduct of refined experiments. Because of the many lines of investigation in different places that these materials have and are being used in, it was necessary that this point be driven home, so that repetition of these experiments or the orientation of others of similar character must have always this point in mind, to either employ the same or identical materials in the operation, or else endless confusion will surely result. (2) The condition determined has considerable value in the insight that it gives into the nature of heterogeneity in nature, of a sort that might well be productive of the initial stages of wide separation of portions of the original stock, and the origin in nature of localized groups that might become the basis of "varieties," "species," or other taxonomic groupings of whose reality there is not the least doubt, and of which the problems of their origin are well worth careful experimental investigation.

In the time following the publication of Darwin's work, the existence of groups in the population of this sort were frequently postulated and rather generally believed in, but I think were not accurately demonstrated in any instance. With the rise of the biometricians the effort to recognize and determine these groups was taken up anew, and with what hopefulness is fully indicated in the writings of the most eminent of the biometricians—Galton, Pierson, Davenport, Dunker and others—but the methods and the tests produced no real progress, save keeping alive the question. The investigations of De Vries and his postulate of "elementary species" presented the old question in a new light, and the pure-line conception of Johannsen added but a new method of analytically isolating some of the possible population conditions upon which divergence and heterogeneity in nature might be based. I believe that the investigation of conditions in the population by the combined methods of biometrics and genetic analysis, as is employed here, may lead directly to the formation in terms of modern conceptions of hypothesis of the methods of species formation that are capable of experimental proof. I shall have occasion to consider these conditions in further experiment and analysis as a basis for the development of conceptions of species formation in nature, without the aid of abrupt and cataclysmic happenings or the long-time accumulation of undeterminate utilitarian "variations."

In this analysis of heterogeneity diverse aspects of many problems have been presented and discussed. I have utilized mainly one set of characters, for the reasons given, and combined in this investigation I used statistical methods, the tests by breeding, and modern analyses of any sort that would elucidate the problems considered.

It is shown that the systems investigated and their component elements, the simplest characters, are the product of gametic agents in the composition of the organic system; that they are specific in position, relations, and in result. It further is evident that the same agent may at different times and in different systems sustain different relations; hence the diversity of the arrangements presented between the elements of the pattern-system, an array that in many respects reminds one of the series presented in many complex inorganic series where the same members are present, but in differing arrangements. It is shown that the system, although complex and the product of many interacting agents, nevertheless acts as a unit in many reactions, passing through the operations of reproduction and crossing in its entirety, or at other times emerging from the reactions changed in relations and arrangements of the elemental simplest characters, indicating altered relations between the conditioning agents. All of this complexity of arrangement and operation is completely submerged by any attempt to treat the conditions in the pattern in the usual quantitative biometric manner.

Thus far in the analysis of the conditions found in a complex array in nature, I have found out some of the things that were there and some of the agents that are productive of the heterogeneity in the system investigated. There appears no evidence for the distinction between quantitative and qualitative that has been drawn by De Vries and Weismann, and the position and rôle or even the existence of the much discussed and abused fluctuation must be questioned and demonstration demanded, and the causes actually determined in a more certain manner, before it is accepted as a constantly present feature of the phenomena of pure lines.

It is clear that it is possible to divide a population into different, homozygous-acting, well-defined biotypic groups that are pragmatic divisions and as such they are in the arrangement of a system, which remain permanent so long as the structure of the system is not disarranged. Within some of these further separations are possible into finer groups, but how many or how far this could be carried is not shown.

In successive generations in the same place and in different places it is clear that there is heterogeneity, that it is delimited and shows in the main determinate response to the total set of conditions, internal and external, that the organism has to meet. It is shown as the result of some of the tests in which populations were transplanted from the different colonies to like conditions in nature or in the laboratory, that the gametic composition of the original colony is a permanent feature of the population at that point. This is important, and one is made to reflect upon how many possible sets of these local permanent differences there may be in a population with a geographic range possessed by many organisms. In my experience it has been certain that in nature it was by no means easy to obtain identical materials from different locations, hence the necessity of the standard locations for the source of the organisms for investigation. My experience with *L. multitæniata, signaticollis, decemlineata, undecimlineata,* and others shows the diversity in the strains from different locations with regard to minor factorial composition in many characters of all kinds.

Any analysis of the situation, as is made here, only gives the state of heterogeneity as it exists at present—some indication of the agents that are operative in the production of the results—but no proof of how the state discovered was

produced in nature. It might seem as if the proper arrangement of experiment and investigation in nature would give the proof of how the heterogeneity arose, but in most of the conditions investigated, even in the simpler ones, the production of a specific end-result is attained through diverse combinations of processes.

At Chalcicomula there was a precisely restricted type of pattern-system which was permanent in the strain. There and elsewhere it never produced the arrangements of the pattern that were found in the population at Chapultepec, especially biotypes 1, 2, and 3. Why did it not produce them? The answer is easy as to why the production of these pattern conditions was not found; there were not in the race the requisite agents in its germinal endowment to produce the reactions that are necessary to bring them into existence. These I could introduce most easily into the race through the medium of crossing. It is probable that some of them might have been produced in other ways without the crossing, and so the "why" of the difference between this and other locations is easy to discover. How did the locations become different? That is another and most difficult question and one that can not, in any instance that I have had to do with, be answered. At Chalcicomula I can never know the source, condition of the original population, the events that have taken place in the colony during its history, any or all of which may be of vital importance in the production of the present condition. From a race taken at some other location I might produce the same conditions by environmental agencies, by elimination, by utilitarian selection; but it is no proof that the conditions in the colony at Chalcicomula arose in the same way and as the result of the same forces. So long as evolution was regarded as a progressive series of events, of monophyletic origins, and dichotomizing schemes of phyletic relationship, there might be some basis for this plausible array of causes and results having necessary relationship. Even upon this basis there is only the poorest of plausible suppositions of cause and effect. I have most earnestly, in this investigation, in numerous instances, made effort to certainly discover the productive agents of conditions found in nature. A successful termination would have been of practical aid in other portions of my problem and otherwise satisfying in being able to determine exactly the antecedent cause of present conditions in a state of nature. In no instance have I been able to eliminate some possible agents, and so restrict possibly the probable efficient factors to a lesser number than at the start, but in no instance thus far have I been able to attain to the desired end of a proof of the actual cause of the conditions observed.

It is possible to determine methods of producing different races—the agents that must enter into the several compositions—and in this it is possible to arrive at an understanding of how in nature the heterogeneity may have been produced in these localized habitudinally different races. In plants and in animals with low migratory capacities instances of the close proximity of nearly related "species" have been described. Classic examples are the Achatinellidæ in the Hawaiian Islands, described by Gulick, and the species of *Partula* discovered by Mayer and recently investigated by Crampton in the Polenesian Islands. These and other instances that exist of remarkably restricted and localized races in nature have been the basis upon which extensive discussions are based. Gulick has presented the combined results of his

investigations in the Hawaiian Islands, with the interpretation of the meaning and causes of the conditions in the population of the Achatinellidæ. In no instance has the cause of the difference between the forms been determined. In these instances, isolation or separation of some sort is assumed to be an active and efficient agent in the production of the result. As far as the data available are concerned, the isolation may as well be the result of the causes that have produced the heterogeneity in the organisms as that it is the cause thereof. A further unguarded assumption is that the conditions in the environment are uniform, and so are not of any moment in the production of the results in the population. It would have been profitable to have measured accurately even some of the factors in the climatic complex in different mountain valleys inhabited by these animals, and determined the identity or diversity therein. I have been very desirous to find some mountain valleys that are identical in their climatic complexes, but have not been able thus far to locate them.

In the analysis of heterogeneity the problems center about the determination of the factorial composition of the character or of the system investigated. This lies at the foundation of the entire investigation, and without this determination the investigation of heterogeneity is meaningless and futile. The problem is for the biologist as for the lithologist, crystallographer, or chemist, the determination, first of the different internal and external agents that enter into the production of a character of the simplest kind, then the relation of these simplest characters in the diverse systems into which they enter. In organisms, as in inorganic materials, these agents or factors are of diverse kinds, in no instance bearers of anything, but determinative of ensuing or resultant reactions and products by their presence or absence in the system at different periods of its reaction.

Having determined the factorial composition of any character or system, one is then in position to experimentally determine the laws of heterogeneity through the influence of external incident agents, by metathesis, by the advent or loss of factors in the system, and so to discover how heterogeneity in nature may be produced, to produce it in experiment, and analytically to determine the factorial differences between groups in nature.

In this the categories of variation must vanish; fluctuations will have to be determined as to cause, whether due to states of stability, impurities, misplaced factors with missing complements or loosely combined in the system and nonproductive of anything but erratic disturbances, or to the action of incident forces. Continuity and discontinuity become terms that are merely descriptive of the results of interactions of the factors, and unit-characters become obsolete. In organisms there are unit-characters no more than in rocks or minerals.

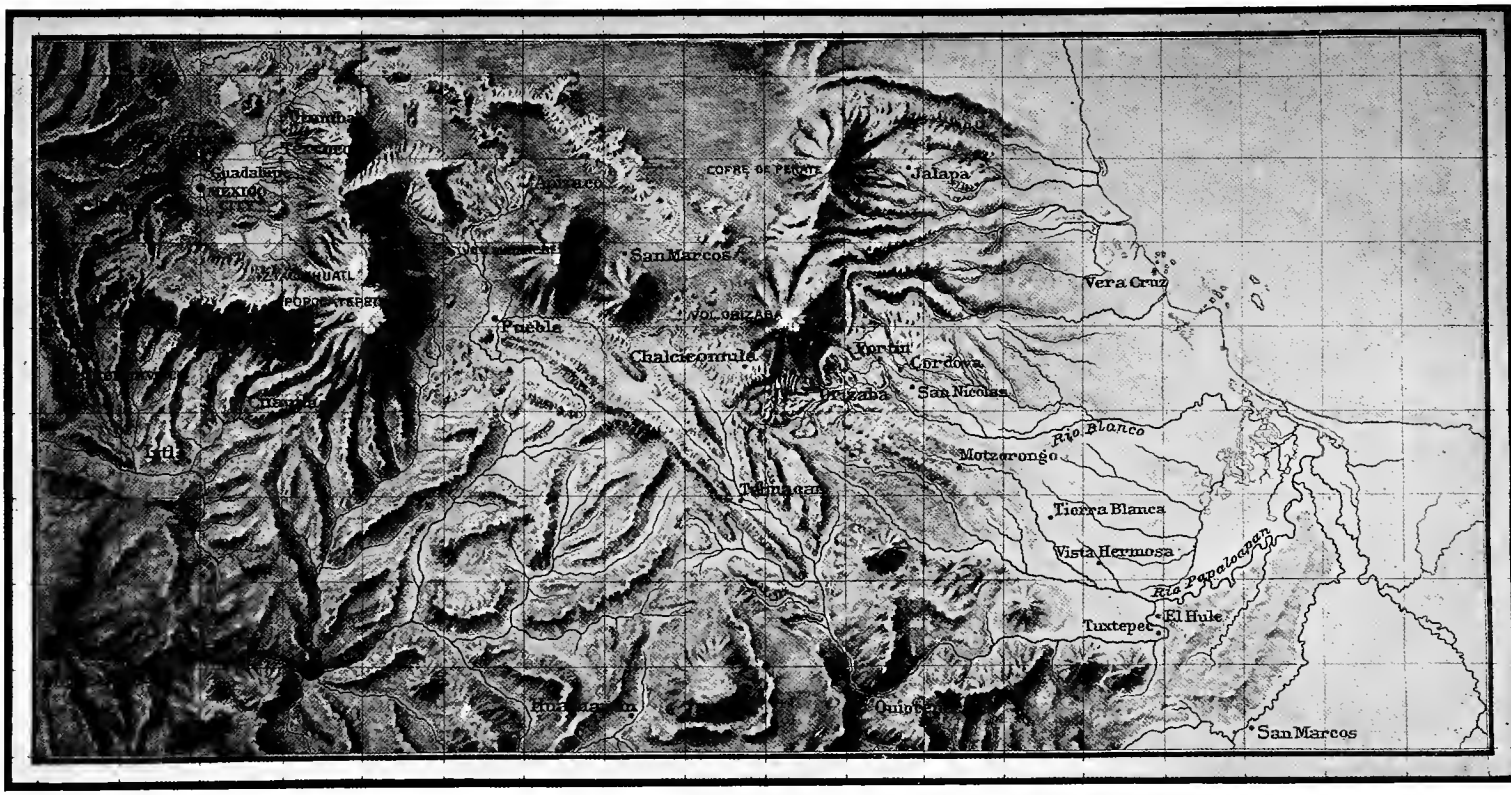
Had we the understanding of the constitution of living substance that we have of the nonliving, it would be easy to isolate and determine the precise nature of any or all factors, to take the entire organism apart and reassemble it in the laboratory. At present no one factor is determined, nor has one been isolated, in a chemical sense.

We thus come to regard the heterogeneity of organisms in a new light, physical in expression, dynamic in action, nonvitalistic in conception. The methods of investigation change from plausible arrangements to analytical determinations of factorial constitution, action, and result, and in experiment, as in nature, the responses in heterogeneity, whether definite or indefinite,

delimited or undelimited, attain increased significance and open up possibilities such that heterogeneity in nature and the origin of natural groups may become a matter of direct experimentation. No doubt methods of their production will be discovered that are capable of being duplicated at any time or place, and no longer will species be the plausible product of adaptations, selections, and survivals of the fittest that have adorned the literature of evolution in the last half century.

The geographic phases of heterogeneity must assume different aspects and the problems become more directly open to analytical investigation, with regard to both internal and external agents.

For me at least the phenomena of heterogeneity in nature take on a different meaning, than when viewed from the one-time aspect of "variation," its categories, definitions, and kinds, utile and non-utile, introducing into the subject and its conceptions elements of causation impossible of interpretation from a physical viewpoint. The conceptions applied in the analysis of heterogeneity in this investigation offer opportunity for unlimited analytical investigation, and in place of biometrics and its plausibilities there are possible determinations of the actual productive agents, their testing, rearrangement into diverse combinations, and the entire removal of the essential factors in heterogeneity and evolution from the situations into which Weismann and others would place them, in inaccessible bearers of characters, operating in an inacessible world of their own. Hope for progress in the future lies only in analytical experiment, and at present the factorial conception of the constitution and evolution of organisms provides the only purely physical, working hypothesis.



MAP OF CENTRAL AND EASTERN PORTION OF MEXICO, TO SHOW POSITIONS WITH REFERENCE TO TOPOGRAPHY IN WHICH THE STUDIES OF POPULATION COMPOSITION WERE MADE.

APPENDIX

THE RELATION OF WATER TO THE BEHAVIOR OF THE POTATO BEETLE IN A DESERT

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THE RELATION OF WATER TO THE BEHAVIOR OF THE POTATO BEETLE IN A DESERT.

INTRODUCTION.

In a series of experiments maintained by Professor Tower to determine the action of the Tucson Desert upon evolutionary processes in chrysomelid beetles, it was observed that soil-moisture, humidity, and the like played an important rôle in modifying the activities of these organisms when introduced into the arid region; so, as a result of these observations, the author undertook a series of investigations to discover any possible connection between this water-relation and the reactions of the potato beetle, *Leptinotarsa decemlineata* (Say), when transplanted into the desert from a temperate habitat.

A large stock of this species was sent to Tucson from Chicago in June 1911, so that comparative studies under different environmental complexes could be made. Three cultures were established in several open-air breeding-cages at the stations already equipped for Professor Tower at Tucson and Chicago. Two of these stations, which were arid in character, are designated as Tucson Station A and Tucson Station B, while the third one, known as the Chicago Station, was temperate and located at the University of Chicago. The former of the two desert stations was situated at the base of the northern slope of Tumamoc Hill, just within the flood-plain of the Santa Cruz River, at an altitude of 2,370 feet, while the latter was located on the shoulder of this hill, on which the Desert Laboratory is situated, at an elevation of 2,705 feet. The biological significance of the conditions at these stations, as indicated, is given elsewhere, and the problems dealt with concern the relations which exist between the activities of the beetles when allowed to reproduce at these localities and the changes produced in the water-content of the animals through the action of the various environmental factors.

INSTRUMENTATION AND CONDITIONS OF EXPERIMENT.

At each station the evaporation rates were obtained by the Livingston atmometers, and Friez self-recording thermographs were employed to measure the temperatures, air and soil, and the same maker's hygrographs were also used for the relative humidities; these instruments were calibrated and standardized fortnightly. The rainfall-readings were obtained from a standard weather bureau rain-gage at the Laboratory site. It is interesting to notice that the environmental data as recorded from these experiments showed for the arid complex that the greatest daily fluctuations occurred at Station A and the highest evaporation at Station B, while the lowest evaporation-rates and air-temperatures were at Station C. The Tucson region as a whole, when contrasted with the Chicago conditions, has a higher rate of evaporation, a lower relative

humidity, a stronger light intensity, and a wider daily fluctuation in both air and soil temperatures; excessive nocturnal radiations and convectional currents were also potent factors in the desert.

Four seasons were apparent in the arid region: A winter rainy season extending from November until April; a dry fore-summer season, from April until July; a midsummer rainy season, from July until the middle of September; and a dry after-summer season, from the middle of September until early in November. At Chicago rain occurred throughout the year. The annual rainfall from several years' data was about 12 inches at Tucson and 30 at Chicago. The monthly records for both stations are given in Table 1 for the three years during which these experiments were in progress.

TABLE 1.

Station.	Year.	Jan.	Feb.	Mch.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Total.
Tucson.....	1910	1.00	T.	T.	0.05	T.	0.22	4.20	4.65	0.62	0.08	1.74	0.06	12.62
	1911	1.31	0.99	0.25	0.27	0.00	0.07	1.57	2.06	2.65	1.23	T.	0.85	11.25
	1912	0.00	0.37	2.12	0.28	0.32	0.61	3.00	0.98	0.01	1.78	0.00	0.39	9.84
Chicago.....	1910	3.07	0.89	0.29	3.84	4.67	0.91	1.79	3.08	3.90	1.79	1.31	1.32	26.86
	1911	1.17	2.27	1.45	3.03	3.37	2.54	2.65	3.72	4.03	3.79	3.27	2.54	33.83
	1912	0.84	1.57	2.20	2.55	3.97	1.78	3.86	3.59	3.26	3.52	1.45	1.08	29.67

NOTE.—The rainfall records at Tucson were obtained from a standard Weather Bureau rain-gage at the Laboratory site. The Chicago data were taken from the records of the Weather Bureau.

MATERIALS.

The potato beetles were collected in May 1911, near Chicago, as they emerged from hibernation, and were allowed to breed there as a group-culture within a large cage filled with potato plants until late in June. A part of this material was then sent to Tucson, where it became the progenitor of the animals used in the majority of the experiments. Organisms when collected from nature are often hybrids, so that crossing of different generations may have taken place, but for complete breeding-records and life-histories of stocks used see Table 2, which shows that these materials reacted homozygously. A brief description of their activities follows.

At Tucson Station A these stocks were received on June 26, 1911, and immediately bred as a group-culture, so that 1,328 adults were produced in 25 days, giving generation I. After feeding upon the potato plants for a few days, these first-generation individuals were bred as a group-culture and produced generation II, numbering 2,312 progeny, in 26 days. Many of the beetles of this second generation provided the materials for a large number of the hibernation experiments which were carried on in 1911, but many of the emerged animals were allowed to hibernate during the winter of 1911–12 as stock for work during the following year. When, in June, water was added to the soil within the cage, the organisms emerged from hibernation as a group-culture, which in 29 days gave generation III, of 1,743 offspring. From this material 50 females and 50 males were mated and allowed to breed at random, giving generation IV, of 4,049 progeny, in 26 days. For the above data, see Table 2.

At Tucson Station B the parent group of 104 adults for this culture was received on July 15 and bred as a group-culture, producing 204 offspring in 25 days, thus giving generation I. As soon as the adults from the first generation appeared they were removed to another breeding-cage, but they immediately burrowed into the ground within the experimental cages and hibernated there until September, when they began breeding and in 21 days produced generation II, of 293 progeny. A few days after emerging from pupation all of the beetles went into hibernation without feeding, where they remained until the following summer, when, on May 31, 7 males and 16 females emerged and bred immediately, giving generation III of 283 adults in 31 days. These were allowed to reproduce as a group-culture, giving in 31 days generation IV of 127 offspring.

TABLE 2.

Year, station, and generation.	Duration of breeding.	Period of oviposition.	First stage larvæ.	Second stage larvæ.	Third stage larvæ.
1911, Tucson A, g. I.....	June 26–July 17	July 7–19.....	July 12–21.....	July 13–27.....	July 17–31.....
1911, Tucson A, g. II....	Aug. 1–18.....	Aug. 4–22.....	Aug. 10–26....	Aug. 16–31.....	Aug. 22–Sept. 1
1912, Tucson A, g. III ..	Autumn of 1911	June 4–15	June 12–21....	June 15–28.....	June 18–30....
1912, Tucson A, g. IV...	July 8–29.....	July 15–29.....	July 17–Aug. 2	July 20–Aug. 5	July 25–Aug. 8
1911, Tucson B, g. I.....	July 15–23.....	July 17–23.....	July 21–29.....	July 23–Aug. 2	July 27–Aug. 6
1911, Tucson B, g. II....	Sept. 3–7.....	Sept. 3–7.....	Sept. 5–11.....	Sept. 7–19.....	Sept. 11–21....
1912, Tucson B, g. III...	May 31–June 20	June 5–20	June 14–26.....	June 16–30.....	June 20–July 10
1912, Tucson B, g. IV...	July 10–Aug. 12	July 24–Aug. 12	July 27–Aug. 14	Aug. 4–27.....	Aug. 12–29.....
1911, Chicago g. I.....	.....	June 6–16.....	.....	.....	.....
1911, Chicago g. II.....	.....	Aug. 1–15.....	.....	.....	.....
1912, Chicago g. III.....	.....	June 2–19.....	.....	.....	.....
1912, Chicago g. IV.....	.....	Aug. 1–15.....	.....	.....	.....

Year, station, and generation.	Pupa state.	Emerged as adults.	No. of adults.	Adults hibernated.	Duration of life cycle.
1911, Tucson A, g. I.....	July 19–Aug. 1	July 31–Aug. 14	1328	None.....	25–26 days.
1911, Tucson A, g II....	Aug. 26–Sept. 2	Sept. 2–13.....	2312	Over winter...	22–29 days.
1912, Tucson A, g. III...	June 21–July 8	July 4–16.....	1748	None.....	28–30 days.
1912, Tucson A, g. IV...	July 29–Aug. 11	Aug. 8–26.....	4049	Oct. 1.....	24–28 days.
1911, Tucson B, g. I.....	July 29–Aug. 8	Aug. 10–18.....	204	Aug. 18–Sept. 3	25–26 days.
1911, Tucson B, g. II....	Sept. 12–22....	Sept. 19–29....	293	Sept. 30.....	20–22 days.
1912, Tucson B, g. III...	June 25–July 15	July 10–23.....	283	None.....	33–35 days.
1912, Tucson B, g. IV...	Aug. 14–31.....	Sept. 1–7.....	127	Sept. 2–8.....	26–36 days.
1911, Chicago g. I.....	.....	July 1–20.....	.....	None.....	25–35 days.
1911, Chicago g. II.....	.....	Sept. 1–10.....	.....	Sept. 1–10.....	25–80 days.
1912, Chicago g. III.....	.....	July 10–12.....	.....	None.....	23–38 days.
1912, Chicago g. IV.....	.....	Sept. 4–20.....	.....	Sept. 20–22....	34–36 days.

Early in September all were in hibernation and remained in the ground during the winter. On the other hand, at the Chicago Station, the original wild parents were allowed to breed as a group-culture, and gave generation I in 30 days. These adults bred as a group-culture and produced generation II in 33 days, which now hibernated during the winter from September until May, when they reproduced and gave generation IV in 35 days: for the above data, see Table 2.

The animals for experiment were reared upon potato plants in cages of uniform size (6 by 6 by 6 feet), with sides of wire-netting, 16 meshes to the inch. These cages were furnished with wooden bottoms (6 by 6 by 3 feet), which were filled with a mixture of equal parts of adobe [1] and sand. This mixture proved to

[1] At Station A, where this soil was obtained, the adobe consisted of a clay loam, which constituted the soil-mass of the river flood-plain. This soil was about 8 to 9 meters deep and rested on sand and gravel. Livingston (1910) found it to have a water-holding power of about 18 per cent of its dry weight. The sand used in all experiments was obtained from an arroyo near at hand and had a water-holding power of about 39 per cent of its dry weight.

furnish favorable conditions for plant growth as well as pupation and hibernation activities.

The condition of the experiment required that a certain routine be repeated each time, and some of the most ordinary methods follow. The rates of evaporation were obtained with the Livingston atmometers, which were cut down to a cone of 50 mm. in length to avoid an error introduced by having shellacked bases. These were standardized on the rotating machine in the Genetics Laboratory at Chicago, and showed after standardization a maximum range of 3 per cent from the normal. The dry weights of the insects were obtained as follows: first by killing them in potassium cyanide, and desiccating them at a constant temperature in a vacuum over concentrated sulphuric acid until the dry weights became approximately constant. The soil samples when collected were placed in glass-stoppered weighing-bottles and carried to the laboratory, where they were weighed and dried at a constant temperature of 100° C. The tropic reactions were tested in the constant-temperature room (18° to 20° C.) and the beetles were exposed in wire-netting tubes (30 cm. long and 5 cm. in diameter). All geotropic reactions were tested in the dark, and if the animals crawled to the top of the tube when held in a vertical position they were considered positive, and if they moved to the bottom of the tube, negative. The phototropic reactions were tested with an ordinary 32 c. p. electric lamp in a constant-temperature room. If the organisms crawled toward the direct rays of light when the tube was in a horizontal plane they were recorded as positive and if they moved away from the source of light as negative.

RÔLE OF WATER IN THE REPRODUCTIVE ACTIVITY.

A striking fact that one observes in desert biology is that a remarkable degree of coincidence is shown between the rainy season and the reproductive period of the animals native to such a region. Corresponding periods of inactivity for such organisms occur during the dry season. In studying this problem, Tower (1906) finds this is true for most of the species of *Leptinotarsa* distributed over the American deserts and similar observations were made by Semper (1881) for several desert forms.

For many years Tower has introduced chrysomelid beetles into desert complexes of Arizona from a wide range of habitats, and the majority of these experiments, which were placed under my care, showed that food, enemies, and the like were not the determining factors in the survival of such organisms, but that in most instances the survival was successful if the proper complex for the reproductive activity was attained.

Frequent observations at Tucson indicated that the optimum breeding activity of the potato beetle was coincident with the highest water-content of the medium, since periods of egg-laying were exactly concurrent with those of rain and with the low rates of evaporation. Therefore, it was important to determine experimentally what relations existed between reproductive behavior and changes of water-content within the medium surrounding these animals.

The literature of the subject contains much data in regard to the effects of temperature upon the reproductive activity, but almost none upon the relation of water to reproduction. In reference to the genus *Leptinotarsa,* Tower (1906) states:

"In both tropical and temperate latitudes, the germ-cells do not develop nor reproduction take place until the conditions of temperature and moisture are favorable. . . . . Likewise, in the northern United States and Canada, *decemlineata* may emerge from the ground in April, but the germ-cells do not begin to grow until the coming of the warm moist days in May or possibly June."

Kammer's (1907) experiments show that *Salamandra* (*maculosa* and *atra*) can be induced in varying ways to lay their eggs, depending in part upon the moisture relation. Jacobs (1909) states for the rotifer *Philodina rosela:*

"The period of maximum egg-production had been preceded by a period of desiccation and furthermore, that each desiccation for any length of time has been followed by an increase in the reproductive activity."

Hennings (1907), working on the bark beetle *Tomicus typographus* Linn., finds that the amount of water present acted as a regulatory factor for such activities. The results of these investigators show that egg-production may be modified through changed water-relations.

At Tucson Station A, comparative study of the environmental records indicated that when the atmosphere had a high water-content, and when the evaporation rates were low, then egg-laying took place most frequently. This was self-evident, for during the breeding of four generations of the stock at this locality egg-laying occurred during the following periods: July 7 to 19, August 4 to 22, 1911; June 4 to 15, and July 15 to 29, 1912, which were exactly coincident with the maximum rainy periods at this station. At Tucson Station B the results were similar to those of Station A, since the periods of oviposition were as follows: July 17 to 23, September 3 to 7, 1911; June 8 to 20 and July 24 to August 12, 1912, which coincided with high humidities and low rates of evaporation. At the Chicago station, however, the evaporation rates were lower and the egg-laying was controlled by other factors. For the desert complex these results indicated that the optimum for egg-laying was reached when the organism was subjected to a moist medium.

Since these conclusions were only probable, it was advisable that they be substantiated by further experiment. Therefore, it was necessary to produce artificial differences in the moisture-content of the medium, and observe its effect upon beetles during hibernation and after emergence.

For the purpose of these experiments, 120 beetles (Tucson A, g. II) were removed from hibernation at 8 p. m. June 19 and were placed in ground-glass stoppered weighing-bottles, so that no moisture could enter. They were removed immediately to the constant-temperature room of the Desert Laboratory, where reactions were tested and all responded positively to light and negatively to gravity. At the same time a sample of the soil surrounding the beetles was taken and found to contain 11.3 per cent of water by weight. The animals were now divided into two lots of 60 adults each (30 of each sex), which were found to weigh 10.007 grams and 9.845 grams, respectively. The first batch was subjected to differences in soil-moisture and its effect upon egg-production observed. In the second case the effect of changing evaporation-rates upon oviposition was also observed.

EXPERIMENTS WITH SOIL MOISTURE.

It was important to determine the effect of a varying soil-moisture upon oviposition in these organisms during hibernation. Tubes used in experiments upon this subject were 30 cm. long and 15 cm. in diameter, and made of wire netting surrounded by several layers of tinfoil to prevent the egress and ingress of moisture. Three of these tubes were filled with a mixture consisting of equal parts of sand and adobe, and then sunk in a large box of sand, so that only the tops were exposed. The sand in the box was kept damp by means of self-watering automatic soil-cups, which were devised by Hawkins (1910), the purpose of this wet sand being to keep the organisms within the tubes at a uniform temperature, a result attained through rapid evaporation of water-vapor from the surface of the soil.

The desired differences in soil-moisture were produced in the above tubes in the following manner: In one tube were placed two porous soil-cups, which gave the soil a high water-content; in the second one, however, a small porous soil-cup was placed, which kept the soil within at a lower moisture than in the former; and in the third, no soil-cup was employed, thus keeping the soil dry. It was thus possible to obtain differences in soil moisture with other conditions approximately uniform. But to make certain that the above apparatus produced the desired results, determinations of soil-moisture within these tubes were made every second day throughout the experiment. These data are tabulated in Table 3, which shows that the moist soil contained 15.8 per cent moisture, the medium moist soil 8.8 per cent moisture, and the dry soil 1.9 per cent moisture. Thermometers were placed in these tubes at a depth of 15 cm. and readings were made at 5 and 9 a. m. and at 1, 5, and 9 p. m. When tabulated, these soil temperatures throughout the test indicated a close agreement for all experimental tubes.

TABLE 3.

Soil samples obtained.	Tube 1. Moist soil.	Tube 2. Medium moist soil.	Tube 3. Dry soil.
	Per cent.	*Per cent.*	*Per cent.*
June 21	15.9	8.9	1.7
June 23	15.6	9.2	2.1
June 25	16.0	8.4	1.8
June 27	15.8	8.7	2.0
Average	15.8	8.8	1.9

The 60 beetles of batch 1 were now divided into three groups, and when tested in the constant-temperature chamber were found to react positively to light and negatively to gravity. Each group was now weighed, group A weighing 3.341 grams, group B, 3.329 grams, and group C, 3.337 grams, respectively. Each group was next buried on June 19 at a depth of 15 cm. in each of the three experimental tubes as indicated, members of A being buried in a moist tube, those of B in one less moist, and those of group C in a dry tube. These animals remained as buried until June 27, 6 p. m., when their weights were again tested in the constant-temperature room, and group A was found to weigh 3.213 grams, group B 2.962 grams, and group C 2.107 grams, respectively.

Thus it was discovered that the beetles of group A from the moist soil showed a loss of only 0.128 gram, while their reactions were, as before, positive to light and negative to gravity. The beetles of group B, however, from the medium moist soil, showed a much greater loss in weight (0.367 gram), although their responses were unchanged, except in the case of 3 which were positive to gravity. The beetles of group C from the dry soil indicated the greatest decrease in weight (1.123 grams), and showed a reversal in their behavior.

After this test the beetles were put into separate cages out-of-doors and allowed to breed under natural conditions. A comparison of the rates of evaporation, obtained with Livingston atmometers when placed within these cages, indicated that the environment was uniform. When the activities of these insects were closely observed, the following results were obtained: Those beetles from the wet soil, whose reactions as previously tested, were still positive to light and negative to gravity, moved immediately upward on the potato plants, and began feeding on the uppermost leaves. This indicated that their activities were normal, and on June 30 eggs were laid. On the other hand, those animals from the medium-wet soil also fed on these plants, but no eggs were laid until July 4. This showed that oviposition was postponed 4 days, but that the dry-soil beetles, whose responses were now reversed, were negative to light and positive to gravity. They immediately burrowed into the ground and remained there until the arrival of the summer rains, July 13, when they emerged and laid eggs on July 15. In this case oviposition was delayed 15 days. An analysis of these results follows:

These experiments showed that differences in soil-moisture produced changes in the water-content of these animals as well as modified their behavior. Since the egg-production was changed, we must conclude that beetles emerging from soils of high moisture-content lay their eggs sooner than those issuing from dry soils. The soil no doubt has played an important rôle in the economy of desert organisms, which are known to respond accurately to environmental changes such as we have described; otherwise many forms would have ceased to exist where they are now widely distributed in desert regions.

EXPERIMENTS WITH EVAPORATION RATES.

The second batch of beetles was used to determine the effect of differences in rates of evaporation upon insects just emerged from hibernation. The apparatus for this experiment consisted of three uniform bell-jars placed over pots of potato plants. Each pot was sunk into adobe soil in the bottom of a vivarium, which was an open inclosure covered with wire netting. One of the bell-jars was provided with 8 atmometers, which, through the evaporation of water-vapor from their surfaces, produced both a high relative humidity and a low rate of evaporation. The second jar was furnished with 2 atmometers, and the evaporation-rate was greater in this jar than in the first; but the third was kept dry, for, since no water or atmometer whatsoever was used, a high rate of evaporation was secured. The food-plants in this test were kept in a normal healthy condition by the use of automatic soil-watering cups placed in the earth near the bottom of the pots. A few preliminary experiments demonstrated that the dry bell-jar in direct sunshine would become several degrees warmer than the more moist; consequently a shade was so placed as to give

closer agreement in temperature readings. A small strip of wire-netting was then fastened around the base of each bell-jar to afford a free circulation of air.

The environmental conditions produced artificially within each bell-jar were measured in the following manner: Thermometers were suspended in each jar and temperature readings were taken five times daily at 5 and 9 a. m., 1, 5, and 9 p. m.; they show a close agreement of the three jars. The evaporation-rate was obtained by means of a single atmometer placed in each jar, and the cubic centimeters evaporated by this instrument were recorded twice each day at 8 a. m. and 8 p. m. In averaging the different rates, it was found that the moist jar showed an evaporation rate of 14.5 c. c. daily, the medium moist 20.7 c. c., and the dry 25.0 c. c., respectively. The results show that the air temperatures within each jar were approximately uniform during progress of the experiment, and furthermore that the anticipated differences in the rates of evaporation were produced in this manner. Thus the environmental conditions for this test were experimentally attained.

Sixty beetles of batch 2 were now divided into three groups of 20 each, and on June 20 were distributed to the jars mentioned above. In the jar with a low rate of evaporation the beetles reacted normally in feeding upon the potato plants, and laid eggs on the third day, June 23, but in the jar with the medium evaporation-rate, the animals, though resting upon the plant leaves, laid no eggs for 9 days, or until June 29. In the third jar, with a high rate of evaporation, which produced the greatest degree of desiccation, they stayed upon the potato vines for 5 days or until June 25, when they entered the ground and there remained until the summer rains began July 11; they emerged, however, from the soil on July 13, and oviposition occurred within 3 days.

These results showed that egg-production was modified by differences in the evaporating power of the air surrounding these animals, and that a low rate of evaporation, coincident with a high water-content, encouraged oviposition, but that a high rate of evaporation, which reduced their water-content, retarded reproduction. So with a high rate of evaporation the beetles were desiccated; their tropisms were reversed and they entered the soil, where they remained until their moisture-content was sufficiently increased; they absorbed water until their reactions became normal, when emergence resulted.

It was shown clearly that reproductive activity occurred during a period of high water-content in the surrounding medium, whether atmosphere or soil, and that desiccation, by reversing the animal's tropisms, inhibited and postponed reproductive reactions of the *L. decemlineata,* which is a typical grassland organism. This demonstrated that the introduced stock had adjusted itself to the complex of desert conditions and reacted in the same manner as did the indigenous organisms of the surrounding region. Accordingly, in the majority of species, we should expect reproduction to coincide with the season of high water-content and a dormant period to follow the dry season, regardless of any discovered structural adaptations.

RÔLE OF WATER IN THE PRESERVATION OF LIFE.

In the desert it was found that hibernating insects could continue life during long dry seasons of one or more years; therefore, experiments were undertaken to answer the following questions: How was such vitality preserved? Was this

relation due to a reduction of normal physiological activities through desiccation? If so, what relation exists between such organisms and changes in the physical composition and especially the moisture-content of the medium surrounding them? For these tests, animals of different physiological activities, induced by differences in humidity, were buried for certain periods of time in soils of varying degrees of moisture and texture. The criteria used in determining the power of resistance were the number of individuals surviving in the test at the end of a given period of time, and the differences in activities of the insects produced through desiccation, as compared to a similar set in which the behavior was normal. The capacity of these animals to resist was tested, and beetles were placed in wire-netting tubes (50 cm. long by 10 cm. in diameter) which permitted a free circulation of air and moisture. The tubes containing the insects were buried upright, so that the base of each was 60 cm. deep in the soil. The plots were 10 meters apart, in the open, at Tucson Station A. Earth was removed from each plot so as to leave two holes 6 meters square by 1 meter deep, and each cavity was further divided into equal parts by a partition of red-wood boards 1 inch thick; one side was filled in with sand, the other with adobe. One of these plots was exposed under natural conditions in the open, while the other was covered with a roof, which extended on each side 1 meter beyond the limits of the plot. This roof was raised 1.5 meters above the ground in order to give a free circulation of air and to keep the soil dry underneath. Water from rains was collected in ditches which conveyed it beyond the plot. The plot in the open was designated Plot A, and that under roof as Plot B. The soils in both plots were kept moist by adding water by means of a garden hose until October 20, but after this date they were exposed to the conditions of the winter of 1911–12 at Tucson.

The first set of experiments concerned only beetles of the summer generation that were emerging from the pupa state. Such insects do not normally hibernate, but may be caused to do so through adverse conditions such as those which cause desiccation. It should be noticed that in one instance the animals were buried with all activities normal; in the other, they were first induced to hibernate in cages in the vivarium, and they were sifted out in the soil; in either case they were finally buried under the conditions described above.

In the former test in which the beetles were buried with all their activities normal, 400 emerging individuals (Tucson A, g. I) were collected on August 12; 50 of these were then placed into each of the 8 wire-netting tubes; 4 of these tubes were filled with sand and 4 with adobe soil; the insects were placed at corresponding levels in each of the 8 tubes; 2 of these containing sand were buried in the open plot and 2 under the shelter, while the 4 tubes with adobe soil were sunk in the adobe sections of the plots, 2 in each. These were left unmolested until May 1, when one tube under each set of conditions was examined but no living beetles were found. On October 1, the remaining 4 tubes were inspected with the same results; there were no living organisms found. These results showed that no beetles of the summer generation with their activities normal hibernated successfully when buried under the conditions of this experiment. These observations are tabulated in Table 4.

In the latter test, however, the insects were first induced to hibernate in cages in the vivarium, and were then immediately sifted out of the soil, to be finally buried in Plots A and B. The following methods were used in this test: During the period of August 13 to 20, emerging adults to the number of 1,613 (Tucson A, g. I) were collected and placed in pedigree cages under adverse conditions within the vivarium. These conditions were produced by having their food reduced to sliced potato tubers and by adding just enough water to keep the soil slightly moist, so that the beetles were partially desiccated. A census taken September 10 showed that 1,209 insects had successfully hibernated in

TABLE 4.—*Census of counts on covered plot and open plot.*

Conditions of the experiment.	Covered plot.				Open plot.			
	Adobe.		Sand.		Adobe.		Sand.	
	Alive.	Dead.	Alive.	Dead.	Alive.	Dead.	Alive.	Dead.
A. Beetles of summer generation:								
1. With activities normal when buried on August 12. On May 1 the following count was made.....	0	50	0	50	0	50	0	50
Another census on Oct. 1 showed...	0	50	0	50	0	50	0	50
2. Others were desiccated and buried on Sept. 10. On May 1 the following count was made.....	44	6	0	50	46	4	4	46
Another census on Oct. 1 showed...	12	38	0	50	0	50	0	50
B. Beetles of winter generation:								
1. With activities normal when buried on Sept. 10. On May 1 the following count was made.....	0	50	0	50	0	50	0	50
Another census on Oct. 1 showed...	0	50	0	50	0	50	0	50
2. Others were desiccated and buried on Oct. 2. On May 1 the following count was made.....	48	2	0	50	45	5	26	24
Another census on Oct. 1 showed...	39	11	0	50	0	50	0	50
3. Others were hibernated and buried on Oct. 7. On May 1 the following count was made.....	49	1	4	46	47	3	39	11
Another census on Oct. 1 showed...	46	4	0	50	0	50	0	50

these cages, and from this result, it was discovered that their vitality was greatly diminished, for 67 per cent of them were killed by these conditions. Of the survivors, 400 were divided into 8 groups of 50 individuals each, which were placed in tubes buried beside the 8 which were described in the former test. All were left unmolested during the winter and until May 1, when 4 tubes from each plot were examined. Tubes from the covered plot showed that those in sand contained no life; 2 from adobe contained 54 living beetles; while 4 were alive in the sand from the covered plot; the adobe portion of this harbored 46 living insects. On October 1 the remaining 4 tubes were also examined; those in adobe soil under shelter contained 12 living beetles, but all were dead in the sand and there were no living animals in either part of the open plot. These experiments proved that beetles of the summer generation, which normally

breed and produce a hibernating winter generation, may be buried after having been induced to enter the ground through desiccation; furthermore, that the animals lived many months when buried under these conditions, but that the death-rate was greater in the sand, and the majority in the open plot succumbed. These data are also given in Table 4.

On the other hand, the second set of experiments concerned beetles of the winter generation which had just emerged from the pupa state. This problem was considered from three aspects: (1) Some animals were buried with all their activities normal; (2) others were induced to hibernate through partial desiccation produced through adverse conditions, and were then buried; (3) many were allowed to hibernate normally before they were finally buried in the two plots.

For the first test in which the animals were buried with all their activities normal, 400 emerging adults (Tucson A, g. II) were collected on September 10; they were placed within the sand and adobe portions of the two plots. During the winter, and until May 1, they were left unmolested, when tubes from each plot were examined and no living individuals were discovered. On October 1 the remaining tubes were exhumed, but no live animals were found. These results showed that, when beetles of the winter generation, which normally hibernate, were buried with all their activities normal, hibernation was unsuccessful and all the animals succumbed (Table 4).

In the second test, where beetles were induced to hibernate through desiccation, 1,000 emerging adults (Tucson A, g. II) were collected on September 11, and were placed under adverse conditions in pedigree cages in the vivarium, where their food was sliced potato, as in a previous test. On October 2, hibernating adults to the number of 692 were sifted from the soil, and 308 dead ones were gathered from its surface; 400 of the living insects were then divided into 8 groups, and were buried within the soils of the two plots, in order to afford the opportunity of winter hibernation. On May 1 these tubes were examined for living beetles; the tube from sand under the covered plot contained no living adults, while in the one from adobe earth were found 48 living adults; also those in sand from the open plot contained only 26 living individuals, while 45 beetles were removed from the tubes in adobe. In a similar manner, on October 1, the remaining 4 tubes were removed. In the adobe soil tube, under the shelter, were found 39 animals, but no individuals hibernated successfully in sand; moreover, tubes from the open plot harbored no life. The results indicate that induced hibernation was effected in the winter generation through desiccation, which increased the resistance of these animals by decreasing their normal activities. The only insects alive at the end of the experiment were found in adobe under the covered plot, which proved that potato beetles, when hibernating in adobe, possessed a greater resistance to desiccation than when buried in sand (Table 4).

In the last test, insects were permitted to hibernate normally, then buried in Plots A and B. This was a control for former tests, since it showed that no error was introduced through handling or digging up the animals. For this test, 1,000 emerging adults (Tucson A, g. II) were collected on September 13; they were placed in a large out-of-door cage that was provided with potato plants, and other environmental conditions were apparently normal. After consuming much food, these animals were in hibernation by October 7; then,

400 of these were sifted from the soil and buried in the adobe and sand portions of the two plots, after having been placed in tubes as in previous tests. On May 1, when 4 tubes from these plots were examined, it was found that the tube from adobe in the covered shelter showed 49 living beetles, while the one from sand contained only 4; on the other hand, the tube from the adobe portion of the open plot was found to have 47 living insects, and those from sand 39. On October 1, when the remaining 4 tubes were removed, those in adobe from the sheltered plot contained 46 live animals, but those in the sand none; those from the open plot contained no individuals which had hibernated successfully. This natural type exhibited the greatest resistance because of the large number of survivals, and it also appeared that adobe was more favorable to the maintenance of life than was sand.

In Table 4 the results are briefly indicated; it is shown there that insects with all activities normal die when buried, for no beetles were found under any of these conditions. It appears also that either the summer or winter generation may be buried and still live, providing the animals were desiccated previous to burial. It is also shown that a covered plot with adobe soil is a most favorable condition for the preservation of life. It is also demonstrated that insects can be desiccated at any time, when they will burrow into the ground, and may remain there many months without apparent injury. These tests further show that the large pores in sand permitted too rapid drying, so that the animals were desiccated beyond recovery. Livingston (1910) shows that this adobe soil has a water-holding power twice as great as sand, which agrees with the above results and explains why these insects continued to live. Lastly, since the adobe soil in an arid region does contain such a high percentage of moisture, it therefore is the best medium for the sustentation of desert life.

RELATION OF WATER LOSS IN INSECTS WHEN EXPOSED TO CHANGES IN THE RELATIVE HUMIDITY OF THE SURROUNDING MEDIUM AND ITS EFFECT ON THE ACTIVITIES OF SUCH ORGANISMS.

The relation of water-loss, *i. e.*, transpiration and respiration from exposed surfaces, to the behavior of plants and animals has already received some attention. This is especially true of plants, the water-relations of which have been studied by Livingston (1906), Lloyd (1912), MacDougal (1912), Renner (1910, 1911), and other plant physiologists. The results of Livingston (1906) are of interest in this connection, since they show that there is a close relation between the daily march of evaporation, as measured by the atmometer, and transpiration in plants. The following experiments upon insects show that these animals exhibit a physiological behavior not unlike that of transpiration in plants, but the results further show that tropisms of insects are modified by loss of water, which in turn is governed by the evaporating power of the air. There is wide literature upon perspiration, but it does not bear directly upon our problem; accordingly we shall consider such researches as have been made upon transpiration and evaporation and the efficiency of these processes in modifying behavior.

The results which follow upon the behavior of insects and other desert animals and upon the relation of evaporation to their behavior and life economy was

reported, by the present writer (1911, 1912), and these results have been substantiated by Shelford (1914 *a*, *b*) and his students, Weese (1917), Hamilton (1917), and Chenoweth (1917). The writer's experiments upon the potato beetle and other desert animals (1911) showed that "the fundamental activities of this beetle, as well as those of many desert organisms, are directly conditioned by their water-content or water-balance. The water-content of the beetle is determined by the evaporating capacity of the air, the leaf-moisture content of its food plant, soil-moisture, and temperature. Variation in any one of these factors may influence not only hibernation, but other habits and reactions." This work was carried on during the next year (1912), in which I stated regarding the behavior of desert animals that "the proportion of water held in the body, or the water-balance, is correlated with various activities, and the lowering of this balance, or surplus, inhibits several functions or processes, and is also followed by reversed response to various external agencies which may exert a stimulatory action."

Shelford (1913) records that certain spiders, ground-beetles, wasps, millipeds, frogs, and salamanders react in consequence of evaporation, and that a short exposure to evaporation conditions increases sensibility to it. Aside from this experimental data, Shelford and Deere (1913) established laboratory methods for determining the reactions of the above animals to evaporation gradients. My experiments differ from Shelford's in being made under natural conditions out-of-doors, while his studies were undertaken in the laboratory.

Hamilton (1917) studied certain soil insects, in the full-grown larval and adult state, of the family Carabidæ, and his results tended to show that an increase in the rate of air-flow did not effect the larvæ as much as did an increase in temperature or a decrease in relative humidity; the adults, moreover, offered greater resistance to evaporation and temperature. The experiments upon the horned lizard by Weese (1917) demonstrated a clear-cut reaction to the substratum temperature gradient, while the evaporation gradient was not the limiting factor. On the other hand, Chenoweth (1917) concludes that the evaporating power of the air is the best index of environmental conditions affecting the white-footed woodland mouse, as well as other land mammals, and that the mice reacted to evaporation whether it was produced by movement, dryness, or heat.

EXPERIMENTS UPON EVAPORATION, TRANSPIRATION, AND BEHAVIOR.

Previous experiments upon *L. decemlineata* show that tropic activities for light and gravity can be reversed through desiccation, and furthermore, that normal reactions are restored if the beetles were surrounded by a moist medium. On the other hand, it seemed important in this connection to perform certain experiments, in order to determine if these insects in nature react to losses of water, which might be produced through desiccation by means of the evaporating power of the air immediately surrounding them. Therefore, it seemed advisable to devise certain tests which would show the daily march of evaporation and transpiration when compared with their behavior.

The first experiment was made to determine the relation between the daily progress of evaporation and transpiration rates of *L. decemlineata* when exposed at three different strata, which were produced by an association of potato plants that completely filled the bottom of a cage, 6 feet square by 4 feet high, and

covered with wire-netting. This dense growth produced horizontal zones with atmospherical moisture, varying from high water-content at the bottom of the cage to one of low content above the plants in the open.

All beetle exposures and environmental measurements were made every 2 hours for a period of 12 observations at 3 strata within the cage, where insects and instruments were exposed within wire-netting tubes, 30 cm. long and 5 cm. in diameter. Stratum A was 5 cm. above the ground near the base of the potato plants, and contained the greatest moisture, thus giving the lowest evaporation rate; stratum B was 60 cm. above the ground, near the center of the cage among the plants, and was directly above stratum A, so that it contained less moisture, which gave a medium rate of evaporation; while stratum C was 90 cm. above ground and 5 cm. above the tops of the plants, and furnished the driest conditions, with a high evaporation-rate, which was the only exposure to true desert conditions. Each stratum was directly above the other, and all exposures were made near the center of the cage. The environmental measurements were obtained as follows: The evaporation rates, by using Livingston atmometers; relative humidities from wet and dry bulb readings; temperatures, from uniform standard centigrade thermometers.

The environmental measurements were made every 2 hours for a period of 12 observations at the 3 strata within the experimental cage as previously described and at the beginning of each period a new batch of beetles was exposed to these conditions for 2 hours. The results are given in table 5.

The beetles used in this experiment (Tucson A, g. II) were collected as soon as possible after their emergence. Since these newly emerged individuals take no food until after 24 hours, all collections were made previous to this time, so that no error might be introduced in consequence of feeding; moreover, no food was given them at any time, and no excretion of waste products by the animals was observed throughout the test. The beetles were placed at once in bell-jars of uniform size in the constant-temperature room, which stood at 24° C., and a high but uniform relative humidity was produced by placing wet filter-paper inside the jars; this kept the air of the jars approximately saturated and the beetles absorbed moisture until their reactions and physiological states were uniform, as was proved by tests made later.

The animals were retained in the jars until needed for further experiment. Three batches of 10 beetles were removed from the constant-temperature room, and exposed every 2 hours in wire-netting tubes, at the 3 strata within the cage. Each batch was made up of similar stocks as follows: 4 individuals of 180 adults which had emerged on July 5 were placed in the constant-temperature room at 8 a. m. July 6; 3 adults of 110 individuals which had emerged on July 6 were also placed in this chamber on July 7; and 3 animals of 124 adults which had emerged on July 7 were likewise placed in this room at 10 a. m. July 8; while a batch of 30 beetles which emerged July 8 received the same treatment at 12 p. m. July 8, and were used during the last 2 hours of the experiment, beginning at 4 p. m. on July 10. Thus all the organisms used were of the same culture and of the same parents; in general they were of the same age, and approximately of similar states, so the conditions of the test were uniform.

Aside from the environmental records, the following was also determined as far as the insects were concerned: the total weight in grams of 10 beetles when exposed, their weight in grams after 2 hours' exposure, the total grams of dry

weight for each batch exposed, the grams of water in the beetles, the total percentage of water in the animals, the loss percentage of water in terms of entire weight, and the loss percentage of water in terms of dry weight (Table 5).

TABLE 5.

Time of observation.	Stratum.	Rate of evaporation.	Air temperature.	Relative humidity.	Total weight when exposed 2 hours previous.	Total weight after 2 hours exposure.	Loss in weight during exposure.	Total dry weight of the solids in the beetles.	Total amount of H_2O in the beetles.	Total H_2O in the beetles.	Loss H_2O of entire weight.	Loss H_2O in terms of their dry weight.
		c. c.	*° C.*	*p. ct.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*p. ct.*	*p. ct.*	*p. ct.*
8 a. m.	A	2.0	26.8	36	1.2957	1.2700	0.0257	0.239	1.0567	81.55	2.43	10.75
	B	2.3	26.8	33	1.2879	1.2574	0.0305	0.235	1.0529	81.75	2.89	12.98
	C	3.6	27.3	30	1.2367	1.2028	0.0339	0.229	1.0077	81.48	3.36	14.80
10 a. m.	A	2.2	27.9	35	1.2435	1.2150	0.0285	0.230	1.0135	81.50	2.81	12.40
	B	3.3	33.2	23	1.2860	1.2405	0.0455	0.275	1.0105	78.42	4.50	16.51
	C	6.0	33.9	22	1.2400	1.1585	0.0815	0.226	1.0135	81.73	8.04	35.98
12 noon	A	2.7	30.8	35	1.1840	1.1496	0.0344	0.223	0.9610	81.16	3.58	15.42
	B	4.6	36.2	19	1.1740	1.1196	0.0544	0.213	0.9610	81.85	5.66	25.53
	C	9.5	36.2	15	1.1870	1.0500	0.1370	0.218	0.9690	81.63	14.14	62.84
2 p. m.	A	4.3	33.3	30	1.2090	1.1320	0.0770	0.232	0.9770	80.81	7.88	33.19
	B	8.4	40.2	15	1.2750	1.1590	0.1160	0.272	1.0030	80.86	11.56	42.64
	C	17.6	40.0	12	1.2140	1.0405	0.1735	0.236	0.9780	80.56	17.74	73.51
4 p. m.	A	4.6	33.9	28	1.2415	1.1610	0.0800	0.229	1.0125	81.55	7.90	34.93
	B	8.9	40.5	13	1.1005	0.9805	0.1200	0.209	0.8915	81.00	13.46	57.41
	C	19.4	40.0	11	1.2540	0.9800	0.2740	0.232	1.0215	81.46	26.82	117.84
6 p. m.	A	5.3	28.3	33	1.2565	1.2015	0.0550	0.215	1.0415	82.73	5.28	25.57
	B	8.1	37.3	15	1.2230	1.1185	0.1045	0.213	1.0100	82.58	10.34	49.06
	C	16.1	37.1	12	1.1965	1.0550	0.1415	0.217	0.9795	81.86	14.44	65.20
8 p. m.	A	4.6	28.4	35	1.2030	1.1815	0.0215	0.210	0.9930	82.54	2.16	10.24
	B	7.6	34.0	19	1.2380	1.1885	0.0495	0.219	1.0190	82.31	4.85	22.60
	C	14.3	33.4	14	1.2780	1.2110	0.0670	0.234	1.0440	81.69	6.41	28.63
10 p. m.	A	3.0	25.4	43	1.2080	1.1845	0.0235	0.213	0.9945	82.32	2.36	11.00
	B	4.6	30.6	24	1.1580	1.1295	0.0285	0.214	0.9435	81.47	3.02	13.28
	C	9.0	30.0	17	1.2565	1.2160	0.0405	0.239	1.0175	80.97	3.98	16.95
12 p. m.	A	2.0	25.0	44	1.2660	1.2375	0.0285	0.228	1.0380	81.99	2.74	12.50
	B	3.1	29.7	27	1.2230	1.1900	0.0330	0.223	1.0000	81.76	3.30	14.79
	C	6.9	29.0	23	1.2055	1.1606	0.0449	0.220	0.9855	81.75	4.55	20.40
2 a. m.	A	2.1	24.1	47	1.1727	1.1420	0.0307	0.224	0.9487	80.89	3.23	13.70
	B	3.2	28.0	31	1.1693	1.1380	0.0313	0.222	0.9473	80.01	3.30	14.09
	C	6.7	27.5	27	1.1384	1.0489	0.0489	0.213	0.9254	81.29	5.28	22.91
4 a. m.	A	1.3	24.3	45	1.1422	1.1122	0.0300	0.224	0.9182	80.39	3.26	13.39
	B	3.1	27.1	28	1.2815	1.2435	0.0380	0.251	1.0305	80.40	3.68	15.13
	C	5.1	27.2	25	1.1390	1.0990	0.0400	0.225	0.9140	80.25	4.37	17.77
6 a. m.	A	1.2	22.3	56	1.4385	1.3975	0.0410	0.301	1.1375	79.07	3.60	13.62
	B	3.1	25.2	33	1.4550	1.4010	0.0540	0.312	1.1430	78.56	4.72	17.30
	C	4.9	25.2	27	1.5375	1.4795	0.0580	0.328	1.2095	78.66	4.79	17.68

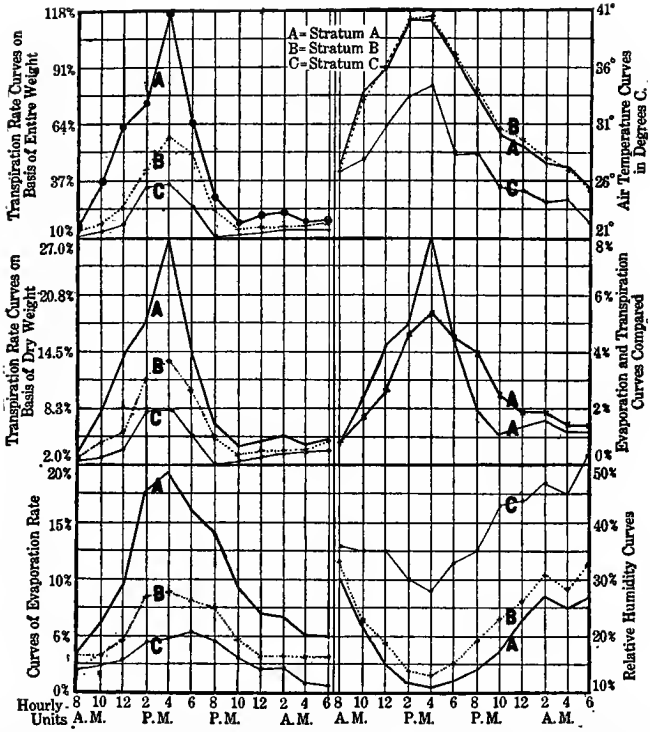
A= Stratum A
B= Stratum B
C= Stratum C
Transpiration Rate Curves on Basis of Entire Weight
Transpiration Rate Curves on Basis of Dry Weight
Curves of Evaporation Rate
Air Temperature Curves in Degrees C.
Evaporation and Transpiration Curves Compared
Relative Humidity Curves
Hourly Units
A.M. P.M. P.M. A.M. A.M. P.M. P.M. A.M.

FIG. 1.—Curves showing relation between daily progress of evaporation and transpiration rates of beetles when exposed to different strata produced by an association of potato plants. Consult table 5 for above data.

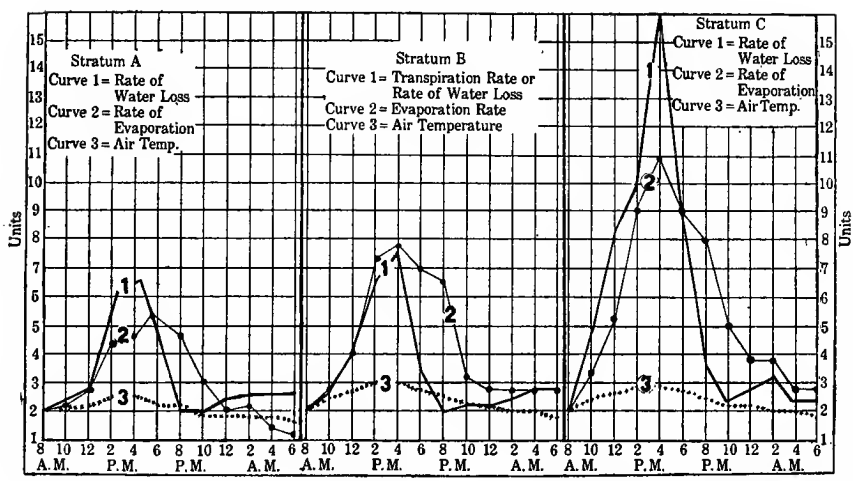
Stratum A
Curve 1 = Rate of Water Loss
Curve 2 = Rate of Evaporation
Curve 3 = Air Temp.
Stratum B
Curve 1 = Transpiration Rate or Rate of Water Loss
Curve 2 = Evaporation Rate
Curve 3 = Air Temperature
Stratum C
Curve 1 = Rate of Water Loss
Curve 2 = Rate of Evaporation
Curve 3 = Air Temp.
Units
A.M. P.M. P.M. A.M. A.M. P.M. P.M. A.M. A.M. P.M. P.M. A.M.

FIG. 2.—Results recorded in table 5 reduced to unity and curves plotted in order to draw a closer comparison than is given in figure 1. The same conclusions are self-evident.

Figure 1 shows the plotted results for the data of this experiment. The broad, heavy lines give the results for stratum A, the broken line, the results at stratum B, and the narrow line those for stratum C. The abscissæ represent the 2-hour time intervals, and each unit along the ordinate represents for the evaporation curves, 2.5 c. c.; for the transpiration curves in terms of entire weight, 3 per cent; for the transpiration curves in terms of dry weight, 13.5 per cent; for the relative humidity curves, 5 per cent; and for the temperature curves 2.5° C. These graphs are interesting in that they show a close agreement between the evaporation-rate and transpiration curve for each stratum. Since the humidity and temperature curves coincide closely for the two upper strata, and the evaporation and transpiration curves for these strata vary in a similar direction, it appears that the rate of loss of water from the animals when exposed to the atmosphere agrees closely with the evaporation rates; *i. e.*, the transpiration curves of these organisms, as Livingston (1906) found with plants, are largely controlled by the evaporating power of the air.

TABLE 6.—*Summary of rate of evaporation in each stratum combined with loss of water from the beetle.*

Location.		Evaporation.		Transpiration.	
Stratum.	cm.	c. c.	Ratio.	Loss per cent H_2O.	Ratio.
A	5	35.3	1.0	47.2	1.0
B	60	60.3	1.7	71.3	1.5
C	90	119.1	3.4	113.9	2.4

Figure 2 shows the water-loss in percentage, the evaporation-rates, and the air-temperatures, all reduced to unity. The broad, unbroken line represents the rate of water-loss in each case; the narrow, unbroken line, the evaporation rate for each stratum; and the broken line, the air-temperatures for each stratum. In making comparisons broadly, the air-temperatures agree in being represented by nearly straight lines, so that they were negligible; but the evaporation curves and curves of water-loss differ for each stratum, yet are similar when compared. At stratum A, the evaporation curve rises more rapidly and higher than the curve of water-loss, and the drop in the evaporation curve is faster than in the curve of water-loss. At stratum B, the curves of evaporation and of water-loss parallel each other until 6 p. m. when the evaporation curve drops more suddenly. At stratum C the increased air-movement is an added factor in the environmental complex, so that both the water-loss and evaporation rates rise much higher, although the temperature curves remain the same. The curve of water-loss at stratum C rises more rapidly and higher than the evaporation curves. The latter drops sooner than the curve for evaporation. This appears to be due to the fact that the beetles are more sensitive to the environmental fluctuations than the porous-cup atmometer, so that difference might account for the lagging effect.

These differences in the rate of evaporation in the three strata and the water-losses are given in Table 6. This table shows that approximately 1 c. c. loss from the cup is equal to 1 per cent loss of water from the beetles exposed.

The evaporation rates here shown gave greater differences within this experimental cage than were obtained by Fuller (1911) for all the plant associations studied. Shelford (1912) uses Fuller's data with tables and compares them with conditions in certain animals, stating that distribution and succession of the animals is clearly correlated with evaporating power of air. By a further comparison with the description of stations, Shelford shows that the evaporating power of the air may be taken in this case as an index of materials, abode, and the like. Since the evaporation ratios existing inside of this cage filled with potato plants are greater than those obtained by Fuller for the stations such as Shelford used, it appeared that *L. decemlineata* reared in such

TABLE 7.

Environmental conditions determined.					*Leptinotarsa decemlineata* subjected to desert conditions.								
Time.	Observed rate of evaporation.	Hourly rate of evaporation.	Air temperature.	Relative humidity.	No. of beetles.	Entire weight of beetles.	Observed loss in weight.	Hourly loss in weight.	Observed loss in terms of dry weight.	Hourly loss in terms of dry weight.	No. positive to light.	Per cent positive to light.	General Remarks.
	c. c.	*c. c.*	° C.	%	*No.*	*gms.*	*gms.*	*gms.*	$\%H_2O$	$\%H_2O$	*No.*	%	
10 a. m.	0.0	0.0	30.2	62	26	3.4850	0.0000	0.0000	00.00	00.00	16	60	
11 a. m.	2.1	2.1	31.4	49	26	.....	.....	.....	.....	.....	23	92	
1 p. m.	6.1	3.1	34.0	32	26	.....	.....	.....	.....	.....	23	92	
2 p. m.	2.7	2.7	34.2	33	26	3.3270	.1380	.0345	15.03	3.76	16	60	
3 p. m.	3.1	3.1	35.0	32	26	.....	.....	.....	.....	.....	21	84	
4 p. m.	3.9	3.9	37.7	32	25	.....	.....	.....	.....	.....	21	84	
5 p. m.	3.2	3.2	35.6	33	25	.....	.....	.....	.....	.....	25	100	
6 p. m.	4.3	4.3	34.2	31	25	3.2365	.0905	.0226	9.86	2.47	25	100	
7 p. m.	2.8	2.8	32.3	35	26	.....	.....	.....	.....	.....	25	100	
10 p. m.	5.2	1.7	28.3	46	25	3.1870	.0495	.0124	5.39	1.35	26	100	
6 a. m.	7.0	0.9	23.7	77	25	3.1490	.0380	.0050	4.14	.52	24	96	
10 a. m.	4.4	1.1	33.2	49	26	3.0726	.0765	.0191	8.33	2.08	24	96	
2 p. m.	14.8	3.7	22.5	32	25	2.9895	.0830	.0208	9.04	2.26	23	92	
4 p. m.	6.0	3.0	32.4	42	25	.....	.....	.....	.....	.....	0	0	Very cloudy.
6 p. m.	*0.0*	*0.0*	*22.8*	*90*	*25*	*(Beetles increased in weight.)*					*11*	*44*	*Absorbed H_2O from the air.*
6 p. m.	*1.3*	*1.3*	*26.8*	*64*	*25*	*3.4230*	*.4335*	*.1168*	*47.22*	*11.81*	*21*	*84*	
9 p. m.	3.0	1.0	25.2	69	25	3.3940	.0290	.0097	3.16	1.05	26	100	
12 p. m.	4.3	1.4	23.4	73	25	3.3716	.0225	.0075	2.45	.82	25	100	
3 a. m.	1.7	0.6	23.6	70	25	3.3540	.0176	.0058	1.91	.64	25	100	
6 a. m.	2.0	0.7	22.7	82	25	3.3295	.0246	.0082	2.67	.89	26	100	
9 a. m.	1.8	0.6	28.8	66	25	3.2880	.0415	.0138	4.52	1.51	23	92	
12 a. m.	6.4	2.1	31.4	41	26	3.1825	.1055	.0362	11.49	3.83	22	88	
3 p. m.	9.7	3.2	33.5	42	25	3.0840	.0985	.0328	10.73	3.58	21	84	0.0706 gm. deducted for 2 died.
6 p. m.	6.7	2.2	31.2	43	23	2.9406	.0730	.0243	8.42	2.81	14	61	
9 p. m.	6.2	2.1	28.0	52	23	2.9035	.0370	.0123	4.27	1.42	16	70	
12 p. m.	3.6	1.2	26.3	62	23	2.8800	.0235	.0078	2.71	.90	17	74	
6 a. m.	*3.9*	*0.7*	*24.3*	*72*	*23*	*2.8935*	*.0135*	*(Beetles absorb H_2O).*			*78*		*Absorbed H_2O from the air.*
6 p. m.	*13.4*	*1.1*	*24.1*	*92*	*23*	*3.2695*	*.3769*	*.0314*	*43.17*	*3.61*	*23*	*100*	

a cage has a great range of adaptability. From the base of the potato plants to just a little above their tops, there existed zones of evaporation of wide extremes. To illustrate, Table 5 shows that from 2 to 4 p. m. at stratum A the evaporation-rate was 4.6 c. c. and loss in water-weight of beetles was 7.9 per cent of their entire weight; at stratum B the evaporation rate was 8.9 c. c. and loss of weight of animals exposed, 13.46 per cent; and at stratum C the rate was 19.4 c. c. and loss in weight of the beetles was 26.82 per cent. This shows the evaporation ratios to be 1.00 : 1.93 : 4.22, and the transpiration ratios to be 1.0 : 1.7 : 3.4. For the whole experiment, the ratios of evaporation were 1.0 : 1.7 : 3.4, while the transpiration ratios were 1.0 : 1.5 : 2.4. In general, these ratios are very similar, showing that there is a direct relation between the evaporation-rates and transpiration percentages, and it answers in part the question already raised: Do

the evaporation-rates, the transpiration curves, and the reaction graphs of these organisms correspond? The following experiments give us a further affirmative response by showing that such a correspondence does exist in the potato-beetle and other insects.

The first experiment was performed out-of-doors at the foot of Tumamoc hill near the experimental cages at Tucson Station A. The beetles which had newly emerged from the pupa state were collected at night. They were kept under saturated bell jars at a constant temperature until morning when they were placed in wire netting tubes. At 11 a. m. on July 19 they were exposed to the desert conditions in the open until 6 p. m. on July 21. The following environmental conditions were determined hourly: the rate of evaporation, the

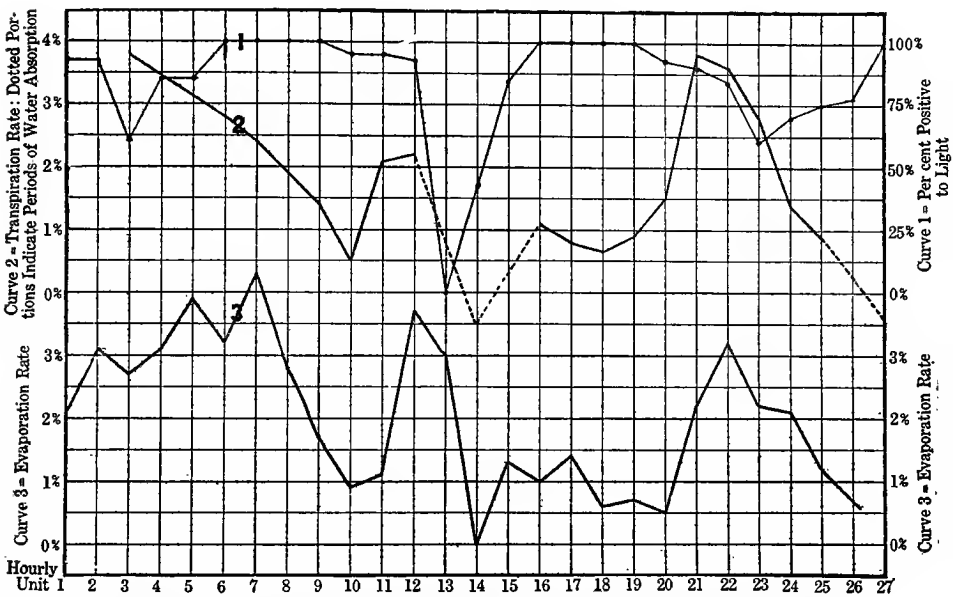
Curve 2 = Transpiration Rate: Dotted Portions Indicate Periods of Water Absorption
Curve 3 = Evaporation Rate
Curve 1 = Per cent Positive to Light
Curve 3 = Evaporation Rate
4%
3%
2%
1%
0%
100%
75%
50%
25%
0%
Hourly Unit
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27

FIG. 3.—Curves 2 and 3 show the relation of evaporation to transpiration (water-loss) of beetles when exposed out-of-doors at the foot of Tumamoc hill; however, Curve 1 shows that the reactions of this insect to light were in general the reciprocal of the evaporation and transpiration curves.

air temperature, and the relative humidity. During this experiment, the entire weight of the beetles was also determined, as well as periods when these insects gave off water to the atmosphere. It is of importance to plant and animal physiologists to state that these beetles absorbed water directly from the atmosphere. Aside from these determinations, others were made, which include the hourly loss in weight of these insects, the observed loss of water in terms of their dry weight, the daily loss of water in terms of dry weight, and the number of the beetles which were positive to light as well as the percentage positive to light. These results are given in Table 7 and the figures in italics in Table 7 show periods when the beetles absorbed water directly from the air. The evaporation rates and the transpiration and reaction rates of *Leptinotarsa* are plotted in figure 3.

By consulting this figure, one can see at a glance that the curves for transpiration and evaporation correspond. Moreover, the reciprocal of the reaction or behavior curve also corresponds to these curves in a similar manner. Thus in the reaction of the potato beetle, its percentages of positiveness was, broadly speaking, the reciprocal of the transpiration curve. This seems to show that the loss of water from the animal, when exposed in the open, determined the reactions of the insect.

TABLE 8.—*Results of subjecting other insects to the same environmental conditions as Leptinotarsa decemlineata.*

Insects.	Time.	No. of insects.	Entire weight of insects.	Observed loss in weight.	Hourly loss in weight.	Observed loss in terms of dry weight.	Hourly loss in terms of dry weight.	No. positive to light.	Per cent positive to light.	General Remarks.
		No.	*gms.*	*gms.*	*gms.*	*% H_2O*	*% H_2O*	*No.*	*p. ct.*	
Catalpa lanigera.	10 a. m.	10	7.2700	0.0000	0.0000	0.00	0.00	9	90	
	11 a. m.	10	.....	.....	.....	.....	....	10	100	
	1 p. m.	10	.....	.....	.....	.....	....	10	100	
	2 p. m.	10	6.9715	.2985	.0746	11.09	2.77	10	100	
	3 p. m.	10	.....	.....	.....	.....	....	10	100	
	4 p. m.	10	.....	.....	.....	.....	....	10	100	
	5 p. m.	10	.....	.....	.....	.....	....	10	100	
	6 p. m.	10	6.7025	.2690	.0675	10.34	2.59	10	100	
	7 p. m.	10	.....	.....	.....	.....	....	10	100	
	10 p. m.	10	6.4970	.2055	.0614	7.90	1.98	0	0	
	6 a. m.	10	6.3620	.1350	.0170	5.19	0.65	0	0	
	10 a. m.	10	6.1785	.1885	.0471	7.25	1.81	0	0	All dead.
Lachnosterna, yellow species.	10 a. m.	16	2.1790	0.0000	0.0000	0.00	0.00	0	0	
	11 a. m.	16	.....	.....	.....	.....	....	0	0	
	1 p. m.	16	.....	.....	.....	.....	....	0	0	
	2 p. m.	16	1.9900	.1890	.0473	22.16	5.54	0	0	3 dead; deduct 0.235 gm.
	3 p. m.	13	.....	.....	.....	.....	....	0	0	
	4 p. m.	13	.....	.....	.....	.....	....	0	0	
	5 p. m.	13	.....	.....	.....	.....	....	0	0	
	6 p. m.	13	1.5715	.1844	.0461	6.21	1.55	0	0	5 dead = 0.4595 gm.
	7 p. m.	8	.....	.....	.....	.....	....	0	0	
	10 p. m.	8	1.0645	.0575	.0144	10.23	2.56	0	0	2 dead = 0.2225 gm.
	6 a. m.	6	0.8030	.0290	.0036	7.30	0.91	0	0	
	10 a. m.	6	.....	.....	.....	.....	....	0	0	All dead.
Lachnosterna, red species.	10 a. m.	8	2.9510	0.0000	0.0000	0.00	0.00	0	0	
	11 a. m.	8	.....	.....	.....	.....	....	0	0	
	1 p. m.	8	.....	.....	.....	.....	....	0	0	
	2 p. m.	8	2.7845	.1665	.0416	18.79	4.70	0	0	2 dead = 0.7525 gm.
	3 p. m.	6	.....	.....	.....	.....	....	0	0	
	4 p. m.	6	.....	.....	.....	.....	....	0	0	
	6 p. m.	6	1.9440	.0880	.0220	14.42	3.61	0	0	2 dead = 0.6005 gm.
	6 p. m.	4	.....	.....	.....	.....	....	0	0	
	7 p. m.	4	1.8065	.0380	.0095	9.84	2.46	0	0	
	10 p. m.	4	1.2705	.0350	.0044	9.07	1.13	0	0	All dead.
Cricket species.	10 a. m.	9	5.1726	0.0000	0.0000	0.00	0.00	5	56	
	11 a. m.	9	.....	.....	.....	.....	....	6	66	
	1 p. m.	9	.....	.....	.....	.....	....	8	89	
	2 p. m.	9	4.8263	.3462	.0866	27.54	6.89	4	44	
	3 p. m.	9	.....	.....	.....	.....	....	4	44	
	4 p. m.	9	.....	.....	.....	.....	....	5	56	
	5 p. m.	9	.....	.....	.....	.....	....	4	44	
	6 p. m.	9	4.6006	.2258	.0565	17.96	4.49	3	33	1 dead = 0.4075 gm.
	7 p. m.	8	.....	.....	.....	.....	....	0	0	
	10 p. m.	8	4.0340	.1590	.0398	14.11	3.53	0	0	All dead.

The second experiment was performed to determine the relation of evaporation to the transpiration and reactions of insects when exposed for several days under natural conditions. To get a comparison between *L. decemlineata* and other insects, a cricket (*Gryllus*), a beetle (*Catalpa lanigera*), and two species of June-bugs (Lachnosternæ) were used, since they could be collected in large numbers. These insects, with the exception of the potato-beetles, were obtained

at night by aid of a light, and were collected as soon as possible after emergence. All were placed at once in bell-jars in the constant-temperature room. A high relative humidity was produced as before by placing a wet filter-paper inside the jars, which permitted the animals, if not already saturated, to absorb water, so that their water-contents would be as uniform as possible, and they would attain, in this respect, a similar physiological equilibrium. The insects were retained under these conditions until 10 a. m. the following morning, when they were exposed in similar cylindrical tubes. The instruments for measuring environmental conditions were also placed in these tubes, which were suspended to a wire and placed in the open, so that each tube was inclined toward the north. The direct rays of the sun were thus permitted to fall upon the tubes at right angles. Unless otherwise indicated in Table 8, the observations were made hourly from 11 a. m. July 19 to 6 p. m. July 21, and for the environmental conditions consult Table 7.

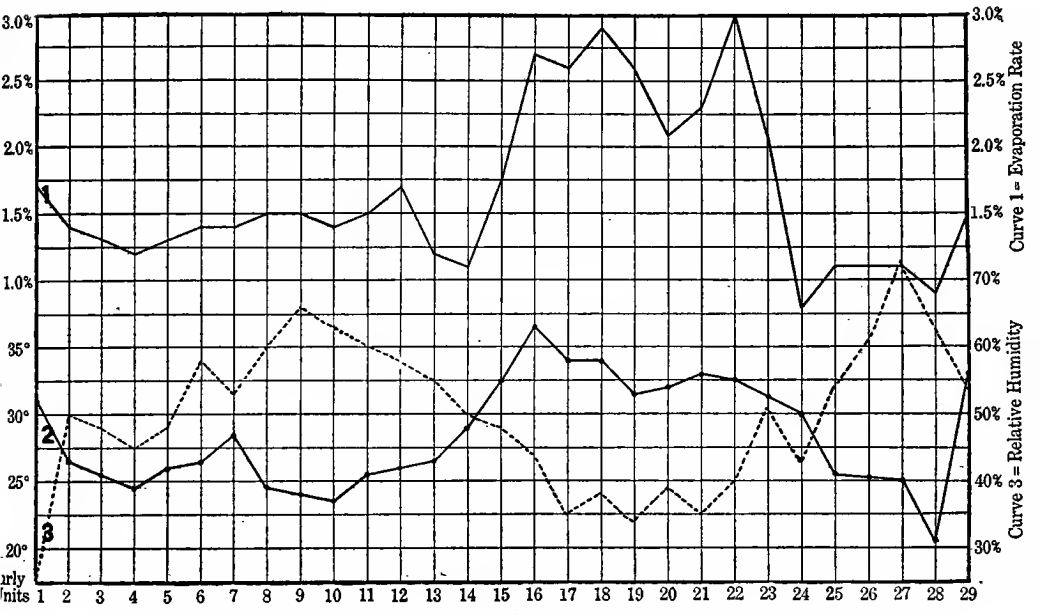

FIG. 4.

If one should plot the results given in Table 8, he will find that all of the organisms used give a transpiration curve which corresponds with their curves of evaporation. *Catalpa lanigera* and the crickets reacted to transpiration in a manner not unlike the potato beetles, while the Lachnosternæ always gave a negative response, regardless of conditions.

Another experiment upon loss of water and insect activity was made as a conformatory test, in which the methods and materials used were similar. The animals consisted of 31 *L. decemlineata* (Tucson A, g. III), 10 *Catalpa lanigera,* and 20 Lachnosternæ. The latter were collected around an electric light and then placed in a refrigerator. All instruments and insects were exposed in the open in large netting spheres and hourly observations were made from 9 p. m. August 9 to 4 p. m. August 11; the complete data are given in Table 9. The evaporation-rates, the air-temperatures, and the relative humidities are

TABLE 9.

Time.	Environmental conditions.						Leptinotarsa decemlineata.							Catalpa lanigera.							Lachnosterna (small yellow species).						
	Rate of evap.	Air temperature.	Dry bulb.	Wet bulb.	Rel. humidity.	Sky.	No. of beetles.	Entire weight of beetles.	Loss in weight.	Loss per cent H_2O in terms of entire weight.	Loss per cent H_2O basis of dry weight.	No. positive to light.	Per cent positive to light.	No. of beetles.	Entire weight of beetles.	Loss in weight.	Loss per cent H_2O basis of entire weight.	Loss per cent H_2O basis of dry weight.	No. positive to light.	Per cent positive to light.	No. of June bugs.	Entire weight of June bugs.	Loss in weight.	Loss per cent H_2O basis of entire weight.	Loss per cent H_2O basis of dry weight.	No. positive to light.	Per cent positive to light.
	c. c.	°*C.*	°*F.*	°*F.*	*p. ct.*			*gms.*	*gms.*						*gms.*	*gms.*						*gms.*	*gms.*				
9 p. m.	1.7	30.8	88	66	31	Clear.	31	3.127	0.000	0.00	0.00	..	..	10	6.734	0.000	0.00	0.00	.	..	20	1.585	0.000	0.00	0.00	.	..
10 p. m.	1.4	26.5	81	67	50	Clear.	31	.....	....	....	.....	10	32	10	.....	....	....	....	2	20	20	.....	....	.....	....	4	20
11 p. m.	1.3	25.4	79	65	48	Clear.	31	3.033	.094	3.48	21.74	22	71	10	6.590	.144	3.30	6.04	4	40	20	1.502	.083	7.55	17.07	4	20
12 p. m.	1.2	24.4	79	64	45	Clear.	31	.....	....	....	.....	12	39	10	.....	....	....	....	5	50	20	.....	....	.....	....	5	25
1 a. m.	1.3	25.7	79	65	48	Clear.	31	2.983	.050	1.85	11.57	9	29	10	6.490	.100	2.30	4.19	2	20	20	1.462	.040	3.64	8.23	2	10
2 a. m.	1.4	26.6	80	69	58	Cloudy.	31	.....	....	....	.....	10	32	10	.....	....	....	....	2	20	20	.....	....	.....	....	1	5
3 a. m.	1.4	28.3	83	70	53	Cloudy.	31	2.926	.057	2.11	13.19	5	16	10	6.443	.047	1.08	1.97	2	20	20	1.412	.050	4.55	10.29	1	5
4 a. m.	1.5	24.4	77	67	60	Cloudy.	31	.....	....	....	.....	18	58	10	.....	....	....	....	4	40	20	.....	....	.....	....	3	15
5 a. m.	1.5	24.0	74	66	66	Cloudy.	31	2.889	.037	1.37	8.56	31	100	10	6.343	.100	2.30	4.19	5	50	20	1.383	.029	2.64	5.97	3	15
6 a. m.	1.4	23.7	75	66	63	Cloudy.	31	.....	....	....	.....	27	87	10	.....	....	....	....	1	10	20	.....	....	.....	....	0	0
7 a. m.	1.5	25.5	77	67	60	Cloudy.	31	2.853	.036	1.33	8.33	28	90	10	6.306	.037	0.85	1.55	0	0	20	1.362	.021	1.91	4.32	0	0
8 a. m.	1.7	26.0	80	69	58	Cloudy.	31	.....	....	....	.....	28	90	10	.....	....	....	....	1	10	20	.....	....	.....	....	0	0
9 a. m.	1.2	26.5	80	68	55	Cloudy.	31	2.797	.056	2.08	12.96	15	48	10	6.242	.064	1.47	2.68	0	0	20	1.323	.039	3.55	8.03	Dead.	
10 a. m.	1.1	28.9	83	69	50	Cloudy.	31	.....	....	....	.....	2	6	10	.....	....	....	....	0	0							
11 a. m.	1.8	32.2	90	74	48	Clear.	31	2.669	.128	4.75	29.63	1	3	10	6.088	.154	3.54	6.46	0	0							
12 a. m.	2.7	36.5	93	75	44	Clear.	31	.....	....	....	.....	2	6	10	.....	....	....	....	0	0							
1 p. m.	2.6	34.0	91	70	35	Cloudy.	31	2.433	.236	8.75	54.63	3	10	10	5.869	.219	5.03	9.19	0	0							
2 p. m.	2.9	34.0	91	71	38	Cloudy.	31	.....	....	....	.....	5	16	10	.....	....	....	....	0	0							
3 p. m.	2.6	31.3	88	67	34	Cloudy.	31	2.393	.040	1.44	9.26	8	26	10	5.723	.146	3.35	6.12	0	0							
4 p. m.	2.1	32.0	89	70	39	Cloudy.	31	.....	....	....	.....	13	42	10	.....	....	....	....	0	0							
5 p. m.	2.3	32.8	91	70	35	Cloudy.	31	2.316	.077	2.86	17.82	15	48	10	5.643	.080	1.84	3.35	0	0							
6 p. m.	3.1	32.4	90	71	40	Cloudy.	24	1.889	.425	7 dead	.....	11	36	10	.....	....	....	....	0	0							
7 p. m.	2.1	29.1	84	70	51	Cloudy.	24	1.821	.088	3.25	20.23	16	52	10	5.545	.098	2.25	4.11	0	0							
8 p. m.	0.8	30.0	86	69	43	Cloudy.	24	.....	....	....	.....	24	77	10	.....	....	....	....	0	0							
9 p. m.	1.1	25.5	79	67	54	Cloudy.	24	1.791	.030	1.43	8.93	11	36	10	5.485	.060	1.37	2.52	0	0							
11 p. m.	1.1	25.0	78	68	61	Clear.	24	1.769	.022	1.05	6.55	0	0	10	5.457	.028	0.64	1.17	0	0							
6 a. m.	6.3	20.5	71	65	73	Cloudy.	24	1.707	.062	2.97	18.45	0	0	10	5.363	.094	2.16	3.94	0	0							
3 p. m.	13.2	33.8	91	75	48	Clear.	24	1.471	.236	18.45	70.23	0	0	10	5.060	.303	6.73	12.71	0	0							
4 p. m.	1.6	32.2	90	76	54	Clear.	24	1.463	.008	0.38	2.38	Dead.		10	5.051	.009	0.20	0.37	Dead.								

shown in figure 4, while the transpiration and reaction curves of *Leptinotarsa decemlineata* and *Catalpa lanigera* are given in figure 5. The upper diagram contrasts for the potato beetle its transpiration rate with the percentage positive to light, while the lower half of the cut does the same thing for *Catalpa lanigera*. This experiment was similar to the former ones, in that the insects were subjected to the environmental conditions out-of-doors at the foot of Tumamoc hill.

For other data and comparisons Table 9 should be consulted; the results given show that the evaporation curve as measured by the porous-cup atmometer and the transpiration curves of the insects are similar, as was previously found to be true. Moreover, the positive reaction curve of the potato beetle was the

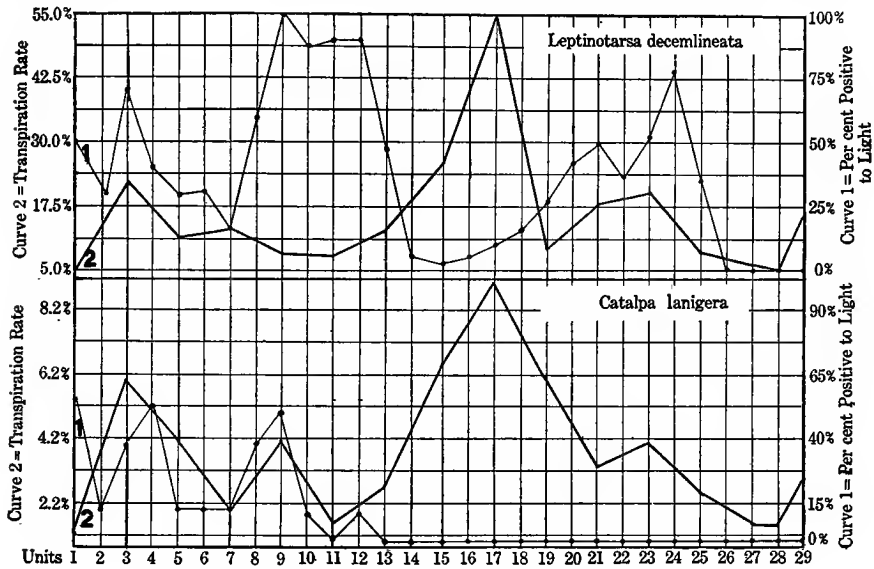

FIG. 5.

reciprocal of its transpiration curve. In the same way the reaction curve of *C. lanigera* was associated with its transpiration curve until 9 a. m., when all reactions became negative. The Lachnosternæ appeared here only with a negative reaction, regardless of their transpiration curve, which agreed with the curve of evaporation.

These results prove, in the first instance, that the evaporating power of the air was the determining factor in the transpiration of these animals, a result similar to that obtained by Livingston (1906) for plants; secondly, that there existed from the base to the top of associated plants, in an arid region, an extreme zonation, in which great differences were found in the evaporating power of the air, and that this in turn controlled the rate of transpiration; and finally, that the evaporating power of the air surrounding the organisms determined their behavior through transpiration. Moreover, many animal organisms of the desert exhibited great localization in their distribution and the ruling feature of the environmental complex, whether it entailed a habitat of trees, among rocks, or in soils, was that of the moisture-relation.

RÔLE OF WATER IN HIBERNATION.

It is an established fact (Tower, 1906) that in the second or winter generation *Leptinotarsa decemlineata* in its homozygous state always hibernates under normal conditions, but that desiccation, or cold, or both, might produce hibernation at any time. At Tucson it was also found that whenever the conditions became adverse enough to produce desiccation, hibernation was produced. Tower (1906) showed that preparation for hibernation in the winter generation consists largely in the reduction of the watery contents of the body and in an elimination of all food and other substances from the alimentary canal.

These facts indicated that the loss of water appeared to be produced by two different mechanisms. One was controlled by an external medium, while the other was determined by heredity. In the former, water was extracted from the tissues through desiccation due to conditions in the medium, while in the latter water was eliminated from the tissues through internal processes under normal conditions. Tower states:

"Preparation for hibernation consists in a physiological change in the constitution of the body for the time being and a consequent lowering of the freezing-point of its tissues in exactly the same way that spores of many plants and the over-wintering eggs of rotifers prepare for the coming of the unfavorable conditions in their environment."

The following experiments were performed to show how the above results can be brought about through desiccation. At Tucson this type of hibernation was quite common, but did not occur in nature at Chicago.

ENTRANCE INTO HIBERNATION.

It is evident that in the potato beetle entrance into hibernation may be "induced hibernation," which occurred whenever the evaporating power of the air surrounding the insect removed more water by weight in a given time than was introduced into the organism by food and other agencies. Such desiccation produced in the course of one or two days depended upon the adversity of the conditions, thus effecting change in the beetle's behavior, so that its reactions were reversed and it burrowed in the soil.

Extensive observations were made at Tucson Station A, where it was discovered that this type of hibernation took place whenever sufficiently desiccating conditions existed. The evidence of such a reaction in a large population was determined by comparing the daily environmental readings with counts of the non-hibernating population, which showed that during the rainy season and as long as water was added to the soil no entrance in this type was found, but when water was discontinued desiccation occurred and hibernation resulted. On the other hand, at Tucson Station B, the "induced hibernation" was always observed as the prevailing type of behavior, since in this habitat the conditions were more adverse. Moreover, at this locality, the growth of the food plants was retarded, since the leaves were tougher and showed less water-content; desiccation was also much greater, so that the response of the organisms to such rigorous conditions was sharper than in any other locality under observation. This clearly demonstrated when a comparison of the daily environmental records were made with the daily count of beetles, which were found out of the

ground during one month of the rainy season. At Chicago, however, no induced entrance was discovered, since the conditions were more favorable there for normal activities, as the daily environmental readings indicate. From these observations it is evident that a type of hibernation occurred during periods of low water-content in the surrounding medium; this produced a lowering of the beetles' content and induced a set of reactions so that a type of behavior, potentially hibernation, resulted; to determine exactly the rôle of water-loss in the observed reactions other experimental tests were performed.

For the purposes of this particular problem, the first test consisted of inducing æstivation by desiccating adults of the summer generation, which do not normally hibernate. The beetles for this experiment consisted of 259 adults (Tucson A, g. III) which had been placed in a culture cage filled with potato plants. They were allowed to feed until July 15, when a few bunches of eggs were deposited, and at 4 p. m. 200 of these beetles were collected and divided into two groups of 100 each, regardless of sex. The beetles were weighed, group A weighing 12.16 grams and group B 11.52 grams, respectively. Group A was now placed under a bell-jar with calcium chloride and group B under a similar jar filled with wet filter-paper. The jars with the beetles were placed side by side in an adobe building under identical conditions, except for differences in the desiccating capacity of the medium within the bell-jars. Throughout the experiment the temperatures ranged from 26° to 38° C. At 8 p. m. July 24 these insects were removed from the soil in the bell-jar and reweighed. Group A from the desiccator weighed 8.53 grams, showing a loss of 3.63 grams, and group B from the humidor weighed 10.92 grams. Previously a box of soil (90 by 60 by 15 cm.) had been filled with a mixture of equal parts of sand and adobe, which also had been already saturated with water and was kept slightly moist throughout the experiment. Two bell-jars (a humidor and a desiccator) were placed side by side over the slightly moist soil in the above box; insects of group A were placed in the latter, and those of group B in the former; after 28 hours group A was in hibernation, but group B did not hibernate, although the beetles remained active upon the filter-paper. The calcium chloride was now removed from the bell-jar over the hibernated group A, and the soil was kept saturated; at the end of 3 days 12 beetles emerged and after 6 days 57 more beetles were discovered, but at the end of 2 days no others had emerged; the soil was then sifted and 31 dead beetles were found. The individuals of group B still remained active, but none, however, had hibernated. These results show clearly that in the summer generation "induced" hibernation with a high death-rate may be produced through desiccation and, furthermore, that when water-balances were restored, all the living individuals emerged again and resumed the activities normal to their generation and season.

To further substantiate the above conclusions other tests follow. In the fall generation, which hibernates normally, 1,000 newly emerged adults (Tucson A, g. IV) were collected on August 14, and divided equally, regardless of sex, into the following four groups: Each group was placed immediately in a separate wire-netting tube in the screened vivarium at Station A, and the tubes were made of wire-netting (95 cm. deep and 35 cm. in diameter), with a similar material covering the top. These were sunk 55 cm. into adobe soil composing the bottom of the vivarium, and each tube was filled to a depth of 53 cm. with a mixture of equal parts of sand and adobe.

The following conditions were experimentally planned in tube 1, containing 250 beetles, to give a low rate of evaporation, and the soil was kept moist by adding water each morning and evening. This tube was kept filled with sprays of *Solanum hertwigii,* which were kept fresh by having the stems in 250 c. c. bottles filled with water, and the sprays were renewed twice daily; it was also necessary to wrap tinfoil about the top of the bottles to prevent the beetles from drowning. The environmental conditions were apparently normal, for the

TABLE 10.

When observed.	Tube 1. Food and moist.				Tube 2. Food and dry.				Tube 3. No food and dry.				Tube 4. No food but moist.			
	Rate of evaporation.	No. of beetles above ground.	No. of beetles hibernated.	No. of beetles dead.	Rate of evaporation.	No. of beetles above ground.	No. of beetles hibernated.	No. of beetles dead.	Rate of evaporation.	No. of beetles above ground.	No. of beetles hibernated.	No. of beetles dead.	Rate of evaporation.	No. of beetles above ground.	No. of beetles hibernated.	No. of beetles dead.
	c. c.				c. c.				c. c.				c. c.			
August 14, 6 p. m.	Set	250	0	0	Set	250	0	0	Set	250	0	0	Set	250	0	0
15, 6 a. m.	1.2	...	..	..	6.0	...	...	...	6.9	...	...	...	1.8	...	..	..
6 p. m.	4.3	...	..	..	21.2	...	...	...	22.7	...	...	...	6.4	...	..	..
16, 6 a. m.	2.0	...	..	..	4.3	...	...	...	5.9	...	...	...	2.8	...	..	..
6 p. m.	6.1	250	0	0	22.0	249	0	1	23.7	241	0	9	8.2	250	0	0
17, 6 a. m.	1.0	...	..	..	4.1	...	...	...	4.9	...	...	...	1.8	...	..	..
6 p. m.	5.7	...	..	..	21.2	...	...	...	24.7	...	...	...	7.3	...	..	..
18, 6 a. m.	1.1	...	..	..	5.0	...	...	...	5.9	...	...	...	1.8	...	..	..
6 p. m.	6.2	250	0	0	19.1	241	7	2	21.8	231	9	10	8.2	248	0	2
19, 6 a. m.	2.1	...	..	..	6.3	...	...	...	6.7	...	...	...	2.8	...	..	..
6 p. m.	6.4	...	..	..	22.2	...	...	...	22.7	...	...	...	9.2	...	..	..
20, 6 a. m.	4.0	...	..	..	7.0	...	...	...	6.9	...	...	...	5.5	...	..	..
6 p. m.	6.8	248	0	2	26.9	124	85	41	23.7	157	19	74	8.2	243	0	17
21, 6 a. m.	2.2	...	..	..	6.3	...	...	...	5.9	...	...	...	3.7	...	..	..
6 p. m.	5.9	...	..	..	25.2	...	...	...	24.7	...	...	...	8.2	...	..	..
22, 6 a. m.	2.1	...	..	..	5.2	...	...	...	5.0	...	...	...	3.7	...	..	..
6 p. m.	4.6	245	0	5	21.3	53	121	76	21.7	133	38	79	7.3	236	0	14
23, 6 a. m.	3.0	...	..	..	4.6	...	...	...	5.0	...	...	...	4.6	...	..	..
6 p. m.	7.1	...	..	..	18.3	...	...	...	20.7	...	...	...	9.2	...	..	..
24, 6 a. m.	4.9	...	..	..	6.3	...	...	...	5.9	...	...	...	5.5	...	..	..
6 p. m.	6.1	241	0	9	24.6	0	157	93	19.8	104	57	89	7.3	221	0	29
25, 6 a. m.	4.0	...	..	..	5.3	...	...	...	5.0	...	...	...	5.5	...	..	..
6 p. m.	5.6	...	..	..	24.2	...	...	...	21.7	...	...	...	7.3	...	..	..
26, 6 a. m.	4.1	...	..	..	6.1	...	...	...	5.9	...	...	...	5.5	...	..	..
6 p. m.	7.0	...	..	..	23.8	...	...	...	22.8	...	...	...	8.2	...	..	..
27, 6 a. m.	2.0	...	..	..	6.0	...	...	...	6.7	...	...	...	3.7	...	..	..
6 p. m.	3.2	222	0	28	18.2	0	157	93	23.0	0	129	121	1.5	177	0	73
30, 8 a. m.	Tubes removed, soil sifted, and all hibernated adults found to be alive.															

water-condition, the food-supply, the low rate of evaporation, and the soil-moisture were all favorable for the normal activities of the animals used (Table 10 for tube 1) in this problem.

The following set of experimental conditions was maintained in tube 2, which contained 250 beetles; however, in this case the soil was kept only slightly moist and the first 5 cm. was used as a dry mulch, so that less moisture was lost through evaporation. The same food plants, *Solanum hertwigii,* were added

thrice daily, but only a few small sprays were used each time, so that the air within the tube was free from dampness, a condition which would have increased the evaporation rate and assisted in making the environmental situation unfavorable. The environmental conditions in this case were normal, in so far as the food-supply was a factor; but the other surroundings were modified, at least as to the high rate of evaporation (Table 10 for tube 2). Both tubes 1 and 2 were planned to give the ascertained differences in evaporation-rates; accordingly tube 1 produced a lower and tube 2 a higher rate, but the food relations for both were approximately normal.

No plants were used in tubes 3 and 4, but each tube was wrapped with several thicknesses of coarse absorbent paper; at the beginning of the test the soil within each was saturated with water, but no water was added during the experiment. Around the outside of tube 4 was placed a coil of lead-tubing drilled full of holes, and this was connected to a carboy of water. This device kept the absorbent paper surrounding the tube saturated and, furthermore, a large piece of oil-cloth was wrapped to the height of 15 cm. around the base of the tube and beneath the absorbent paper. This oil-cloth was extended out in all directions for about 60 cm. from the bottom of the cage, so that the dripping water did not come in direct contact with the soil in the tube. On the other hand, no water was added to tube 3, so that this cage was kept dry during the experiment. These conditions, therefore, produced a high rate of evaporation in tube 4 and a low one in tube 3 (Table 10). The results for each of these tests follow.

The 1,000 beetles used in this experiment were grown under the same environmental conditions and from the same parents, and, moreover, these animals had emerged as adults from the pupa state synchronously; so they were then as nearly uniform physiologically as it was possible to obtain them. The conclusions showed for tube 1 with plenty of food and moisture, when the individuals were counted at the end of the experiment, that there was no hibernation; 28 were found dead upon the soil. In tube 2, with plenty of food, but in which the air was kept dry, the census, when taken at the end of the test, showed that 157 had successfully hibernated and that 93 had died. At the end of the experiment tube 3, which contained dry air and no food plants, showed 129 beetles in hibernation and 121 dead ones. Tube 4, which was supplied with moisture but with no food, at the end of the test gave no evidence of hibernation; 73 of the 250 beetles originally present were found dead upon the soil. These results proved that newly emerged adults of the fall generation can not be caused to hibernate under normal conditions, but that if the surrounding medium was dry a type of hibernation reaction did result through desiccation. Such deductions are possible, since the evaporation rates in Table 10 show that a low rate retarded hibernation and a high one accelerated this behavior.

It is also true that this entrance into hibernation may be of the normal type, which always occurs under normal conditions in the winter generation. Low temperature, however, was an important factor at Chicago, but this kind of hibernation also took place in the pure winter-generation stock, even under high temperatures. This behavior was further studied in the second generation of the year under the following set of experimental conditions.

At Tucson Station A this type of hibernation reaction in beetles of the winter generation (Tucson A, g. II) was observed to appear under a normal environmental complex in the fall of 1911, but all other hibernations (Tucson A, g. IV),

which took place under adverse circumstances in the early fall of 1912, were of the induced type. At Tucson Station B this behavior was not discovered in either the winter generation of 1911 or that of 1912, since the environmental conditions always produced desiccation in the early fall at this locality, thus causing the beetles to be in hibernation about 10 months each year; they emerged about the middle of July, and after feeding for a short period re-entered hibernation late in August. At the Chicago Station, however, normal hibernation always occurred, because the environment was normal, for no excessive desiccation or any other climatic adversities appeared. It became necessary, therefore, to determine if these results could be confirmed by further data, so the following hibernation tests were carried out.

For these tests 30 emerging adults (Tucson A, g. II) on September 2 were placed in a hibernating pedigree-cage containing potato plants for food; the soil was a mixture of equal parts of sand and adobe, and water was added twice daily, but the plants completely filled the cage. The experimental conditions, therefore, were apparently normal throughout the test. It was discovered by daily observations that these animals were in hibernation on September 18 and when dug up on October 2, the first adults were uncovered at a depth of 20 cm., but the larger number of beetles were found at the bottom of the pot. The beetles were inactive when first removed, but began to move in a few minutes at an air-temperature of 33° C. Various tests in the field demonstrated that they possessed no reactions to food or dry soil, but within 5 minutes they did respond, and all burrowed into the moist earth at a temperature of 21° C. This indicated that a cool moist soil accelerated the entrance reaction. These results were further tested to determine if similar reactions always took place.

On September 2, 130 emerging adults (Tucson A, g. II) were put into a hibernating cage, which had been previously filled with potato plants, and in which the soil consisted of a mixture of equal parts of adobe and sand, to which water was added twice daily; thus the conditions were approximately normal, for no desiccation occurred. The beetles responded to this set of conditions, for they were in hibernation by September 22. When dug up on October 21 the insects were found distributed through the soil from the top to the bottom of the cage, but when tested in the field showed no reaction to food or dry soil, and when brought into contact with cool moist soil, out-of-doors, they burrowed into it within 5 minutes.

This activity was again tested in the following manner: 51 emerging adults (Tucson A, g. II) were removed September 3 and were placed in a hibernating cage filled with *Solanum hertwigii* for food. In this experiment a different food plant was also used, but with no apparent result upon their behavior. The soil was also of equal parts of sand and adobe, and water was added each morning and evening, so that the experimental conditions were apparently normal. All the animals were in hibernation by September 19, but when dug up on October 3 only 46 adults were alive. The first individuals, however, were discovered at a depth of 29 cm., and the majority were at the bottom of the pot. When tested in the field no reactions to food or dry soil were observed, but when brought into direct contact with cool moist earth all burrowed into it immediately. Thus when normally hibernating beetles of the winter generation were removed from hibernation a cool moist soil was necessary to initiate this behavior.

ACTIVITIES DURING HIBERNATION.

It was observed that hibernating beetles were inactive when first dug from the soil, and if the insects were moved to a warm room they soon began to crawl; then entrance into hibernation occurred if they were brought into contact with moist earth. At Chicago, during the winter of 1911-12, it was observed that beetles which had hibernated out-of-doors migrated more deeply than usual during the cold winter. From the following test it appeared that beetles would move to moist regions in the earth during hibernation, for late in April 1912, at Tucson Station A, several hundred individuals were found to be hibernating in the open air cage. Accordingly a corner of this cage was watered and the soil was sifted; thus, all the beetles were removed in that locality, but the newly dug soil was kept moist, and each week it was examined for adults; during each observation a large number was always discovered.

WATER RELATION OF SOILS AND HIBERNATING BEETLES.

The beetles hibernate in small cavities or cells, which contain air of a relative humidity, that is in proportion to the water-content of the surrounding earth. During heavy rains the soil becomes flooded with water, so that some air is driven from the cavities, but if the rain continues for too long a period the beetles may die. On the other hand, if the soil is too dry, desiccation of the insects takes place and death may result; therefore, the part soil-moisture plays in mortality during hibernation is of great significance. Tower (1906), in discussing results with soils, states:

"The water-content of soil is controlled, not by an abundant rainfall, nor by telluric water, but almost wholly by adhesion and capillarity in the soil—that is, physical conditions alone, such as permeability, capillarity, and the power to absorb and to retain water are the factors which influence the moisture content of soil . . . . In all soils the pores which do not contain water are filled with air in which the percentage of relative humidity is controlled by the amount of water in neighboring pores. Likewise, the cells in which these beetles pupate are filled with air; the relative humidity is controlled by water in the pores of the surrounding earth."

Thus the physical composition of the soil is important in preserving insect life, and the adobe soil of the Tucson Desert, although it contains but little moisture, does possess other physical potentialities which act in retaining moisture, for through drying it becomes impervious to water and hibernating animals are sealed up in their cells and thus preserved from desiccation. To determine how dry the adobe soil was when containing living beetles, samples of it were taken from the walls of the cells (Table 11) and those of June 30

TABLE 11.

Date.	Depth.	Wet soil.	Dried soil.	Water-loss.	Water-content.
	cm.	*gms.*	*gms.*	*gms.* H_2O.	*per cent* H_2O.
April 1.....	25	60.93	56.18	4.75	8.45
April 8.....	25	122.22	110.00	12.22	11.11
April 30.....	25	123.84	115.89	7.95	6.85
	5	58.94	57.64	1.30	2.25
June 30.....	10	76.55	73.55	3.00	4.07
	20	58.09	55.45	2.54	4.45

showed an average of less than 4 per cent water of their dry weights. This was during the dry season, when no beetles had emerged from hibernation up to the above date, but on July 1, the day following, 0.98 inch of rain fell; 73 beetles emerged on July 2, and by July 3 eggs were laid. This sharp response in behavior must be attributed to the water-content of the soil, for the beetles emerged immediately after the first rain and oviposition took place within 48 hours. The insects of the surrounding desert showed a similar response, inasmuch as the adobe soil held them imprisoned until the first rain, which raised their water-content and softened the soil so that emergence of immense numbers occurred.

The following experiment was performed to show the relation of physical composition of soils to mortality during hibernation. Three hibernating cages were prepared containing soils, one was composed of sand, another of adobe, and a third of equal parts of sand and adobe. On October 1, 500 hibernating adults were caused to hibernate artificially in each tube by placing the insects at a depth of 40 cm. in the soil, and the soil within each tube was kept slightly moist until late in October; they were then allowed to remain out-of-doors under natural conditions during the winter until May 1, when water was added to each cage. On May 3, 362 adults emerged from the soil mixture, 251 from the adobe, but none from the sand. On May 5, the soil of each cage was sifted, when it was found that all in the pure sand were dead but that only 27 in the adobe and 23 in the soil mixture had succumbed. It appeared, then, that there was a relation between soil composition and mortality during hibernation, for the sand permitted excessive desiccation to occur, the adobe and mixed soils did not admit of great desiccation, but most individuals were hibernated successfully in the mixture of equal parts of adobe and sand.

EMERGENCE FROM HIBERNATION.

The physiological complex of emerging beetles was next considered in reference to two phases of the problem. In one case the reactions of hibernating beetles caused to emerge by applying water to the soil were determined, and in the other the reactions of similar adults, in which emergence was produced by sifting the soil, were tested. In the first experiment hibernating beetles (Tucson A, g. II) were encouraged to emerge by applying water on June 1, when they were removed to the constant-temperature room; their reactions were found to be positive to light, but negative to gravity. The light response was further tested by placing 5 beetles in each of 10 test-tubes and each tube was placed so that one-half of it was in the shade of the laboratory roof and the other half in direct sunlight. The beetles all oriented and moved out into the sunlight at the end of each tube, where they remained, and in a few minutes were dead. The air-temperature in the exposed ends of the tubes was 57° C. In this experiment the organisms reacted to sunlight and the suggestion that possibly the red or blue rays might have influenced this result led to the next test.

Of 50 hibernating individuals (Tucson A, g. II) emerged after adding water on June 3, 25 were put in each of two test-tubes; one of which was placed in direct sunlight under a red bell-jar containing a potassium-bichromate solution, and, while no deaths occurred, no definite reaction was observed; the other was placed in the direct sunlight beside the former, under a blue bell-jar provided with a solution of copper sulphate, when all became positive to the rays and

none died. We may therefore conclude that death in the first experiment was due to heat.

Many tests of various kinds have been given elsewhere and with the same result—that when hibernating beetles were caused to emerge by applying water, they reacted positively to light and negatively to gravity. The next experiment consisted in testing the reactions of hibernating beetles, in which emergence was attained by digging and no water was added to the cage.

For this test 47 hibernating beetles (Tucson A, g. II) were removed by digging at 8 p. m. on June 12. They were negative to a 32 c. p. lamp, but 23 of these insects were positive to candle-light of a weak intensity. These were immediately placed under a bell-jar containing moist filter paper, and when tested on June 13 they were found to be positive to light but negative to gravity. This behavior was repeated in the following case.

Twelve hibernating beetles (Tucson A, g. II), when removed by digging at 7 p. m. on June 20, were found to weigh 1.2573 grams, but they gave no response to light or gravity. (A soil-sample taken from the earth surrounding the beetles showed that it contained water to the extent of 12.7 per cent of dry weight.) When the animals were placed in a humidor at 10 a. m. on June 22, they weighed 1.3057 grams, having absorbed 0.1484 gram of water from the moist chamber; 10 of these beetles, when tested, were positive to light, but 2, which were inactive, died in a very short time after the experiment. The 10 adults were also negative to gravity, for when placed in the constant-temperature dark-room, all crawled to the top of the cylindrical wire-netting tube. These experiments showed that positive phototropic and negative geotropic reactions were induced in hibernating beetles by increasing the water-content of the surrounding medium, because the beetles under the moist bell-jar increased in weight and imbibed water directly from the moist air. It was also true that they absorbed water from the air in the soil. This relation was further shown in the following observations, which were made upon the emergence response.

The time of emergence is controlled by the environmental complex, for if water were added to the medium surrounding hibernating beetles, when the soil temperatures were above 14° to 16° C., emergence resulted. This was evident at Tucson, for no emergence was discovered at either station until the rainy season in July, and furthermore, the winter rainy season caused no emergence because of low temperatures. At Chicago, emergence occurred whenever the soil-temperatures reached 14° to 16° C., for enough precipitation always took place during the winter and spring months so that emergence occurred as soon as the proper temperature relations existed, which was from May 20 to June 25.

SUMMARY AND DISCUSSION UPON THE RELATION OF WATER TO HIBERNATION.

The conclusions arrived at from these previous results indicated that a type of hibernation might be produced at any time through desiccation, except with low temperatures, when little desiccation took place. This condition produced a loss of water from the beetles in such a way that they responded negatively to light and positively to gravity, so that they burrowed into the soil and remained there until the moisture-content of the soil was sufficiently high. They then absorbed hydroscopic water, which raised their water-content and reversed their reactions, so that they became positive to light and negative to gravity, hence

their emergence, so that now, in case other conditions were suitable, they were ready to enter upon their reproductive activities. Below 12° to 15° C. in soil-temperature the water-relation was not the controlling factor, but the duration of the hibernating period depended upon the length of the dry season in an arid complex and upon the length of winter (low temperature) in a temperate region.

Baumberger (1914) reviews, at length, the literature on hibernation of insects and reaches the following conclusions, chiefly from his own researches:

"1. That temperature is but a single factor and not necessarily the controlling one in hibernation.

"2. That hibernation is usually concomitant with overfeeding and may be the result of that condition or the result of accumulation of inactive substances in the cytoplasm of the cell due to feeding on innutritive food.

"3. That the loss of water which is general in hibernation probably results in a discharge of insoluable alveolar cytoplasmic structures which have accumulated and produced premature senility with an accompanying lowering the rate of metabolic processes.

"4. That starvation during hibernation, together with loss of water, may result in rejuvenation, when aided by histolysis, and an increase in permeability.

"5. That this rejuvenated condition and increased permeability will, if stimulated to activity by heat, permit pupation in codling-moth larvæ, which in this case is the termination of the hibernating conditions."

The results of Sanderson and Peairs (1913) add another condition for hibernation that was also discovered by Tower (1906) for the potato-beetle; this is the influence of heredity. The former authors reached the following conclusions:

"That our first work was an effort to show that emergence from hibernation was due to an accumulation of temperature, but it soon became apparent that hibernation is very largely controlled by the influence of heredity, and that the relation of the temperature and inheritance must be determined for each species."

For the Mexican cotton boll weevil, Hunter and Hinds (1904) found that dryness was more desirable for hibernation and that mortality during hibernation was greater from exposure to moisture than from cold; but, on the other hand, high temperatures and moisture were the best conditions for the development of such beetle larvæ. In this connection, Baumberger (1914) stated:

"The effects of ether on plants is similar to hibernation and since the action of ether is probably a drying one, this may throw light on the importance of moisture in hibernation."

Loeb (1906) says:

"The lack of water acts similarly to a low temperature. This is the reason why seeds can be kept alive so long. Lack of water may reduce the reaction velocity of the hydrolytic processes in seeds at ordinary temperature so considerably that it may become practically zero."

The snail, according to the results of Kühn (1914) loses weight in winter and reacts to drought in summer. Unless it contains a large amount of water, no dry food is taken, and if placed under moist conditions when in hibernation it will come out of its closed shell. Bellion (1909) finds that a low moisture-content of the air is the determining hibernating factor in the European snail,

and Baker (1911) shows that snails during dry seasons form an epiphragm; they usually bury themselves during hibernation and æstivation. On the other hand, Pearl (1901) finds that the terrestrial slug *Agriolimax* can hibernate in cold water.

In the vertebrates, Rulot (1901) determines for the bat that the proportion of water increases during hibernation from November to April; but there actually was a loss of water, more in proportion at the end than at the beginning. Polimanti (1904) finds that an increase in humidity increases the pounds in a marmot during hibernation.

In conclusion, the work of Sanderson (1908) agrees most closely with my results upon hibernation. In discussing the relation of temperature to the hibernation of insects, he states:

"In come cases, however, the time of emergence from hibernation is controlled by moisture conditions as well as temperature, or independent of temperature. Thus Tower kept the potato beetle in hibernation for 18 months at a high temperature but with a dry atmosphere, and they emerged as soon as normal moisture conditions were produced. Webster and Hopkins have shown a similar effect of the lack of rainfall on the emergence of the Hessian fly in the fall. In relation to hibernation in humid climates the matter of moisture is probably not a controlling factor, but undoubtedly has the most important influence upon the time of emergence of forms in æstivation during the summer or in an arid region."

My results upon the potato-beetle substantiate the work of Sanderson.

EFFECT OF CHANGES IN WATER-CONTENT UPON ALTERATIONS IN TROPIC ACTIVITIES.

The experiments and observations upon *L. decemlineata* proved that, when surrounded by a moist medium, the beetles were positive to light and negative to gravity. It is also evident from previous tests that if the moisture of the surrounding medium was decreased, desiccation resulted, so that the insects were reversed in their behavior and reacted negatively to light and positively to gravity. These beetles, however, responded to any intensity of light if moved from a lesser to a greater intensity, and accordingly when moved from darkness into the moonlight at Tucson they always reacted; and in many instances insects which were negative to a strong light were also positive to a weak one.

It was shown by Burdin (1913) that heat and dryness stimulate positive reactions in terrestrial amphipods, while cold, moisture, and quiet favor negative reactions. The results of Dice (1914) prove that light of high intensity makes daphnias positively geotropic, but a decrease in light intensity has the reverse effect; and furthermore, these animals tend to become positively geotropic in high temperatures and negatively geotropic in low. Kanda (1916*a*), in studying geotropism in a marine snail, found that it is negatively geotropic, but most individuals would orient positively if placed on a dry glass or wooden plate. Later, Kanda (1916*b*) demonstrates for fresh-water snails that they are negatively geotropic when their lungs are empty and positively geotropic when their lungs are full of air. Olmsted (1917) finds that food is a factor in the reversal of the behavior to gravity in *Planaria maculata*. Adams (1903) concludes that earthworms retreat into their burrows during the daytime because of their negative phototropism, but they emerge at night not so

much because of darkness as because of their positive phototropism for faint light. Wilson (1891) shows that *Hydra* is negative to bright light and positive to dim light. According to McGinnis (1912), *Branchipus serratus* is positively geotropic in light and negatively geotropic in darkness; darkness rather than light may furnish the stimulus to this reversal. In studying the reactions of *Drosophila* to gravity, Cole (1917) finds that the response to gravity is much less marked in flying than in creeping, where it is very definite.

Many animals orient in the field in relation to the center of gravity of the earth. Loeb (1905) found that some animals turn their heads upward and others downward. To this latter class belongs the garden spider, which he found may hang in this position in the center of its web for hours. He discovered the same behavior in some diptera. Shelford (1917) states that such animals as the grasshopper usually orient with the head up, while aphids and katydids orient with the head down. In the potato beetle the majority of larvæ and a large number of adults orient with the dorsal side down.

There is a vast amount of literature dealing upon reversibility in phototropism through chemical agencies. Loeb (1893 and 1904) proves that it was possible to reverse the reactions of a large number of water forms through chemicals such as salts, acids, and the like. According to Moore (1912*a*, 1912*b*, and 1913), phototropism in *Daphnia* and *Diaptomus* may be influenced through the agency of caffein, strychnin, atropin, acids, alcohol, and ether. Moore (1913) says:

"While negative phototropism in *Diaptomus* can be reversed by acids, but positive phototropism brought about by chemical means can not be reversed by strychnin (atropin or caffein)."

Wolfgang (1912) determines that electrolites influence phototaxis, and Kanda (1914) reversed geotropism in *Arenicola* larvæ by means of salts.

EXPERIMENTS UPON THE RÔLE OF WATER IN GEOTROPISM.

On May 15, at 8 p. m., 21 freshly emerged beetles (Tucson A, g. I) were moved to a constant-temperature room and tested 10 times as to their reactions to gravity, and in each test all were negative. These geotropic reactions were tested in the dark, and if the beetles crawled to the top of the tube, when held in a vertical position, they were considered as positive and if they crawled to the bottom as negative. The thermograph tracings showed a constant temperature of 21° C., with a daily variation of 1° C., throughout the test. Again at 11 a. m. on May 16, when tested as previously (10 times), they were still negative to gravity, and at this time weighed 2.0009 grams; furthermore, on May 17 at $10^h\ 30^m$ a. m., they weighed 1.9294 grams, and again gave the same test to gravity, so that these results proved that the beetles under these conditions were uniform for this reaction. For experimental purposes these insects were divided into three groups. The first group of 7 adults was put into a calcium-chloride chamber, which produced so high a rate of evaporation as to desiccate them; the second of 7 individuals was subjected to a low rate of evaporation by placing wet filter-paper under the bell-jar, so that little water was removed from them under these conditions; in the third chamber 7 adults

were used as a control. In table 12, the results are given, which shows that group 1 at the beginning of the test was negative, but by 9h 30m a. m., while under the dry bell-jar, all became positive, but when moist conditions were restored in the jar, by 10 a. m. on May 26, they were again negative. In group 2, at the beginning, all were negative and remained thus as long as they were kept under moist conditions, but at 10 a. m. on May 30, all had become positive. In

TABLE 12.—*Reversal in the potato beetle to gravity.*

Date and hour of observation.	Air-temperature.	Group 1.			Group 2.			Group 3.		
		Weight of beetles.	Positive to gravity.	Negative to gravity.	Weight of beetles.	Positive to gravity.	Negative to gravity.	Weight of beetles.	Positive to gravity.	Negative to gravity.
	°C.	*gms.*	*p. ct.*	*p. ct.*	*gms.*	*p. ct.*	*p. ct.*	*gms.*	*p. ct.*	*p. ct.*
May 17, 10h 30m a. m.....	20	0.692	0	100	0.640	0	100	0.597	0	100
May 19, 12 30 p. m.....	20	.573	80	20	.783	0	100	.590	0	100
May 21, 9 30 a. m.....	20	.496	100	0	.801	0	100	.586	0	100
May 22, 10 00 a. m.....	20	.577	25	75	.810	0	100	.577	0	100
May 24, 10 00 a. m.....	20	.682	15	85	.810	0	100	.571	0	100
May 26, 10 00 a. m.....	20	.684	0	100	.703	0	100	.570	30	70
May 28, 10 00 a. m.....	20	.685	0	100	.594	15	85	.563	100	0
May 30, 10 00 a. m.....	20	.683	0	100	.556	100	0	.560	100	0

NOTE.—In group 1 the conditions in bell-jar were dry on May 17, 19, and 21, and moist on the other days. In group 2 said conditions were dry on May 26, 28, and 30, and moist on other days. In group 3 said conditions were uniform throughout.

group 3, which were the control individuals, a gradual loss of weight occurred until 10 a. m. on May 28, when all were positive. These results showed clearly that reactions to gravity may be reversed through changes in the moisture-content of the surrounding medium.

RELATION OF TEMPERATURE TO THE OUTGO AND INTAKE OF WATER.

An interesting discovery was the determination that there was little absorption of water below 12° C. as was shown in a test in which beetles emerging from pupation were collected on July 28; they were placed under a bell-jar containing wet filter-paper, where they remain until 12 midnight on July 30, when equal numbers of insects were placed in two bell-jars, in a refrigerator, at a temperature of 10° to 13° C. One bell-jar contained wet filter-paper and the other calcium chloride, but weighings made at frequent intervals showed that in the humidor there was no appreciable loss during the 84 hours in the refrigerator. In another test, desiccated beetles were placed under saturated bell-jars in the refrigerator, but weighings made at frequent intervals gave no evidence of water-absorption. These results showed how much organisms were protected from absorbing water during winter rains, which would otherwise result in their freezing, and further demonstrated that desiccation, occurring slowly at a low temperature, was a factor in the economy of the organism.

A similar result was obtained with upper temperature limits. It is known that the coagulation temperature of colloids varies with the amount of contained

water, a condition with which our results on *L. decemlineata* agreed generally, since the death-point in potato beetles with a high water-content was 58° to 60° C., and desiccated ones withstood 1° to °5 higher temperature. Bachmetjew (1902) shows that the temperature of the insect's body varied with the conditions, namely, moisture, temperature, and the like. If the air was damp, the body-temperature was higher than that of the surrounding medium, since no evaporation occurred; but if the air was dry it cooled through evaporation. He also pointed out that the smaller the percentage of fluids in a unit of the living insect body, the lower was the normal congealing-point of the fluids. Tower (1906) states that soil-temperatures taken on the savannas of Vera Cruz in April 1904, in places where *L. decemlineata* was æstivating, were frequently as high as 60° to 65° C., and that success in passing through these high temperatures at the end of the dry season depended upon the completeness of the physiological changes preceding entrance into hibernation. These results were similar to those just given for this insect, which showed that the lower and upper temperature limits were influenced through water-relation.

In studying longevity in insects, Baumberger (1914) shows that the temperature at which colloidal substances coagulate lowers with a decrease in water-content and that long exposure to cold or high temperature may result in this decrease in water. He explains that the result of a long exposure to cold is the same as short exposure to heat, while intensity of cold shortens the length of the period. He also demonstrates that the point of coagulation varies with the water-content of the insects studied. Greely (1901) concludes that:

"A reduction of the temperature and a loss of the water have similar effects, because the cell loses water when the temperature is lowered, as well as when the concentration of the surrounding medium is raised."

The results of Livingston (1903) show for *Spirogyra* that a cell loses water when the temperature is lowered. In discussing the reversal in animal instincts, Loeb (1900) concludes that a decrease in temperature has the same physiological effects as a loss of water.

METABOLISM AND THE WATER-RELATION.

The results of various workers show that desiccation modifies the rate of metabolism; thus, the alterations in the behavior of the potato beetle may be due to differences in metabolic activity, brought about through combined relations of water and temperature of the organism. Shelford (1913) states that the changes in activity of the animals used in his experiments were due to the withdrawal of water.

It is known that anything which disturbs the rate of metabolism in an animal alters its response to a stimulus, and that reversed reactions in behavior are caused by changes in this metabolic process. According to Jennings (1904), Child (1910), Wodsedalek (1911), Allee (1912), Phipps (1915), and others a stimulus may change the physiological state of an animal, which produces a modified type of reaction. Many depressing agents are also known, such as potassium cyanide, chloretone, and a low oxygen content. Baumberger (1914) shows that starvation is an agent of this character, since it decreases metabolism by removing material to be oxidized. Loeb (1906), Mast (1911), Shelford (1914), and others further demonstrate that acids and alkalis increase irri-

tability. The results of Shelford (1914) and of Chenoweth (1917), however, agree most closely with those which are recorded in this paper. Both of these observers conclude that a high rate of evaporation increases sensibility or irritability through loss of water, a condition which might account for the alterations in the reactions of the potato beetle.

GENERAL DISCUSSION UPON THE RÔLE OF WATER IN LIVING THINGS.

In order to show why such a large number of reactions in the potato beetle were controlled by its water-relation, it was considered necessary to review only literature which bore directly upon these studies. "It was assuredly not chance," to quote Henderson (1913), "that led Thales to found philosophy and science with the assertion that water is the origin of all things." He also states that the action of water now appears to be a momentous factor in geological evolution, and the physiologist has found that water is invariably the principal constituent of living organisms. Thus, according to this observer, water makes up from 70 to 85 per cent of fishes, about 87 per cent of oysters, 85 per cent of apples, 78 per cent of potatoes, and 95 per cent of the edible portion of lettuce. It is interesting in this connection to add that my results upon the potato beetle show it to contain 80 per cent water. Henderson further says that the organism itself is essentially an aqueous solution in which are spread out colloidal substances of vast complexity, and as a result of these conditions there is hardly a physiological process in which water is not of fundamental importance. According to Livingston (1903), it is absolutely essential that every living mass of protoplasm be saturated with water, since vital phenomena occur solely in aqueous solutions.

Physiologists have long recognized that water is of the greatest importance for normal activity of tissues and that all exchanges of material, all supplies of food, and metabolic processes in general are dependent upon it. Aberhalden and Hall (1908) assert that water is absolutely necessary as a solvent for numerous compounds, for it brings into play various chemical reactions, which take part in building up and breaking down substances without number; it is also a carrier of nourishment to the body and provides the means for the removal of its waste products. In discussing the physical importance of water, Hammarsten and Mendel (1911) show that water by its evaporation is an important regulator of temperature. Davenport (1897) states that growth is due chiefly to imbibed water, and Estabrook (1910) also demonstrates that growth in paramœcium is due almost solely to inhibition of water. MacDougal (1912), Lloyd (1905), and others demonstrated that many plants absorb water directly from the air. In respect to this subject, in animals the frog has perhaps received most attention, and according to Hill (1908) frogs take up water through the skin; they do not drink, for a thirsting frog with its gullet tied increases in weight no less than one with the gullet open. He also states that a frog can be gradually dried to less than 39 per cent of its normal weight without fatal results. My own data for the potato beetle show that it can be desiccated to less than 50 per cent and still live, while *Catalpa lanigera* will die if reduced by 25 per cent and the June bug if dried less than 15 per cent of its normal weight.

It is not true that all animals do absorb water, for my experiments upon the scorpion and horned lizard (*Phrynosoma cornutum*) of the Tucson Desert indicated that these animals would not imbide any aerial water, and even when immersed in it no absorption was detected; furthermore, when desiccated no difference in weight was observed. A large scorpion lived for more than 2 months in a desiccator without food, but it probably died of starvation. If lizards do not absorb any appreciable amount of water or lose any through desiccation, then such a condition might demonstrate why they are distributed in a desert as well as in a hot humid region; therefore, the water-relation would not be the determining factor in a lizard's habitat, but the temperature-relation should be of greater importance in determining its distribution. This might also account for the results of Weese (1917), since he studied the reactions of the horned lizard to evaporation and temperature gradients, but found that the lizard responded definitely to temperature, while there was no marked reaction to evaporation. In this connection the work of Matthews (1913) is important. He says:

"There is a mechanism for rendering mammals tolerably independent of the moisture content of their environment, a mechanism most highly developed in the reptiles. A mechanism formed by the replacing of the wet skin of the amphibian by a dry or scaly skin; the perfecting of the kidneys to maintain osmotic pressure of the blood; the control of the sweat glands and loss of water by the intestines; the development of membranes non-permeable to salts so that animals may sit in fresh water and lose their salts. . . . . By this improvement reptiles have secured almost complete independence of the water-content of their surroundings."

Water is essential to life, says Babcock (1912), for during the period of development it is the most abundant constituent of living organisms. He continues:

"Some of this water is imbibed directly, some of it is taken with solid food which is rarely dry, and some of it is formed within the organisms by metabolic changes in the organic constituents of the food and tissues, induced by respiration and other vital processes. The relative amount of water derived from each of these sources depends upon the kind of organisms, its period of growth, the nature of its food, its environment, and its activities."

His experiments show that many varieties of insects, such as the clothes-moth, the bee-moth, and the flour-beetle, the flour-moth, and others live during all stages of development upon foods containing less than 10 per cent of water. He concludes that nearly all the water used by insects feeding upon air-dried foods is metabolic. In my own experiments upon the potato-beetle and other animals there are no data upon metabolic water and its relation to behavior.

The results of Hegner (1916) further illustrate the water-relation in animals. He arranged an experiment upon oviposition in the potato-beetle, so that 35 batches of eggs were in the sunlight and 15 were in the shade. Those in the sun came to nothing, but all in the shade were hatched. It was found that development had started in the sunlight, but that desiccation probably arrested this process; therefore he concludes that the advantage of concealment is not so great as that secured by shielding the eggs from the desiccating properties of the sun. My results show that the majority of adults orient to gravity with their dorsal side down, which might explain why eggs are usually deposited on the under side of a potato-leaf.

SUMMARY AND CONCLUSION.

In general the results indicate for the potato-beetle that:

(1) The optimum breeding activity of this insect coincides with the highest water-content of the atmosphere, since periods of oviposition are exactly concurrent with those of rain and low rates of evaporation.

(2) Differences in soil-moisture produce alterations in the water-content of these animals which modify their behavior, since beetles will lay their eggs sooner if they emerge from a soil of high moisture-content than if they issue from a dry soil.

(3) Egg-production is also modified by differences in the evaporating power of the air which surrounds these insects, since a low rate of evaporation encourages oviposition.

(4) The beetle dies if buried when all its activities are normal, but either the summer or winter generation may be buried without injury if previously desiccated.

(5) The adobe soil of the arid region retains a relatively high percentage of water and is thus an excellent medium for the sustentation of the life of this beetle, as in other desert animals.

(6) These insects exhibit a physiological behavior not unlike that of transpiration in plants; but further, their tropisms are modified by loss of water, which is governed by the evaporating power of the air.

(7) The evaporating power of the air surrounding these insects determines their behavior through transpiration; even their responses to light and gravity are controlled by evaporation.

(8) Entrance into hibernation in a desert region may be produced at any time through desiccation, except at low temperatures, when little desiccation takes place.

(9) The hibernating period in an arid region is controlled by the duration of the dry season, but is dependent upon the length of the winter in a temperate region.

(10) The water-relation is the controlling factor in the emergence of this insect from hibernation if the temperature is above 15° C.

(11) When surrounded by a moist medium (above 15° C.), either atmosphere or soil, these beetles are positive to light and negative to gravity, but desiccation reverses this behavior.

(12) This insect absorbs very little water below 12° C.; its death-point under a high water-content was found to be 58° to 60° C., but when desiccated it can withstand from 1° to 5° C. more of heat.

(13) Alterations in the behavior of the potato-beetle may be due to differences in metabolic activity as influenced through the water-relation.

(14) This animal also imbibed water directly, but no studies were made upon metabolic water.

We may conclude that *Leptinotarsa decemlineata,* when introduced from its grassland habitat into an arid region, is equilibrated immediately with respect to its surroundings, especially in regard to its water and temperature medium; that its behavior is changed to resemble those responses still present in an organism long accustomed to a desert complex, and that, since water is the

limiting factor in an arid region and the prime essential for metabolism, the behavior of the potato beetle in a desert is determined chiefly by the water-content of its environment. Henderson (1913) states:

"Water, of its very nature, as it occurs automatically in the process of cosmic evolution, is fit, with a fitness no less marvelous and varied than that fitness of the organism which has been won by the process of adaptation in the course of organic evolution. . . . In truth, Darwinian fitness is a perfectly reciprocal relationship. In the world of modern science a fit organism inhabits a fit environment."

These results upon the potato beetle indicate that its marvelous fitness and adaptation to water is such a "reciprocal relationship."

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